UNITED STATES DEPARTMENT OF AGRICULTURE
MISCELLANEOUS PUBLICATION NO. 636

Washington, D. C. Issued November 1947

BUTTER AS A SOURCE OF VITAMIN A IN THE DIET OF THE PEOPLE OF THE UNITED STATES

By

THE TECHNICAL COMMITTEE IN CHARGE OF THE NATION-WIDE SURVEY

United States Department of Agriculture, Agricultural Research Administration, Bureau of Dairy Industry and Office of Experiment Stations, in Cooperation with the Agricultural Experiment Stations of Alabama, Arizona, California, Idaho, Indiana, Iowa, Kansas, Louisiana, Maryland, Minnesota, Mississippi, Montana, Nebraska, New York (Cornell), North Carolina, Ohio, Oregon, Pennsylvania, Washington, Wisconsin, and Wyoming

UNITED STATES
GOVERNMENT PRINTING OFFICE
WASHINGTON : 1947

For sale by the Superintendent of Documents, U. S. Government Printing Office
Washington 25, D. C. - Price 15 cents

FOREWORD

In August 1941 the Committee on Food and Nutrition of the National Research Council, recognizing that butter produced under winter-feeding conditions is usually much lower in vitamin A potency than butter produced under summer-feeding conditions, transmitted a proposal to the Secretary of Agriculture recommending that attention be given to the development of feeding methods for improving the vitamin A potency of milk and butter during the winter. The Committee recommended further that studies be undertaken to determine the normal variation in the vitamin A values of milk and butter, as affected by seasonal and regional differences in feeding practices, and to determine also the vitamin A values of butter as sold on the retail markets. The Committee wanted the information in order to be able to estimate how much vitamin A the people actually get from the milk and butter they consume.

The Secretary forwarded the recommendation to the Bureau of Dairy Industry and the Office of Experiment Stations, Agricultural Research Administration. Representatives of these Department agencies outlined a plan of procedure and presented it to the directors of the State agricultural experiment stations during the annual meeting of the Association of Land-Grant Colleges in Chicago, in November 1941, with the result that a Nation-wide cooperative survey was approved.

The directors of the State experiment stations designated C. H. Bailey, Director of the Minnesota Agricultural Experiment Station, to work with O. E. Reed, Chief of the Bureau of Dairy Industry, and James T. Jardine, Chief of the Office of Experiment Stations, as a committee to develop cooperative plans for the study, to assume over-all administrative direction of the project, and to appoint a Technical Committee to formulate the analytical methods and procedures.

The Technical Committee was composed of the following scientists: L. A. Maynard, New York (Cornell), chairman; C. J. Koehn, Alabama; H. R. Guilbert, California; F. P. Zscheile, Jr., Indiana; G. W. Snedecor, Iowa; L. S. Palmer, Minnesota; I. L. Hathaway, Nebraska; W. H. Peterson, Wisconsin; and C. A. Cary, Bureau of Dairy Industry. Later, C. A. Cary was made chairman, W. D. Salmon of Alabama succeeded C. J. Koehn, and W. F. Geddes of Minnesota succeeded L. S. Palmer.

The first responsibility of the Technical Committee was to organize and undertake such cooperative work as was necessary to develop suitable methods for obtaining samples of butter and for determining its carotene and vitamin A content. Meantime, plans were developed and arrangements were made for participation by State experiment stations that were so located as to be able to provide data fairly representative of the butter produced in the United States.

After a year's cooperative work, the Technical Committee made recommendations regarding methods of obtaining butter samples and determining their vitamin A potency. These methods were approved by all agencies participating in the study and, in all essential respects, these were the ones used in the analytical work and in compiling the results. The Technical Committee worked closely with the State leaders throughout the study and also assumed the responsibility for the compilation and evaluation of the data.

The study itself and the organization necessary to undertake it were complex and the work was carried to completion with difficulty under wartime conditions. The organization, procedures, and results will stand as an example of what can be accomplished by voluntary cooperation.

W. V. Lambert

W. V. Lambert,

Administrator, Agricultural Research Administration.

UNITED STATES DEPARTMENT OF AGRICULTURE

MISCELLANEOUS PUBLICATION No. 636

Washington, D. C. Issued November 1947

BUTTER AS A SOURCE OF VITAMIN A IN THE DIET OF THE PEOPLE OF THE UNITED STATES

By the Technical Committee in Charge of the Nation-Wide Survey[1]

United States Department of Agriculture, Agricultural Research Administration, Bureau of Dairy Industry and Office of Experiment Stations, in Cooperation with the Agricultural Experiment Stations of Alabama, Arizona, California, Idaho, Indiana, Iowa, Kansas, Louisiana, Maryland, Minnesota, Mississippi, Montana, Nebraska, New York (Cornell), North Carolina, Ohio, Oregon, Pennsylvania, Washington, Wisconsin, and Wyoming[2]

CONTENTS

[1] Members of the Technical Committee were C. A. Cary, Bureau of Dairy Industry (Chairman), W. F. Geddes, Minn., H. R. Guilbert, Calif., I. L. Hathaway, Nebr., W. H. Peterson, Wis., W. D. Salmon, Ala., G. W. Snedecor, Iowa, F. P. Zscheile, Jr., Ind.

[2] The project leaders in the various cooperating agencies were as follows: W. D. Salmon, Ala.; R. N. Davis, Ariz.; G. A. Richardson and M. L. Long, Calif.; D. R. Theophilus and O. E. Stamberg, Idaho; B. E. Horrall, Ind.; E. W. Bird, Iowa; J. S. Hughes and W. H. Martin, Kans.; E. A. Fieger, La.; C. W. England, Md.; L. S. Palmer, W. F. Geddes, and Robert Jenness, Minn.; Marvin Gieger, Miss.; J. A. Nelson, Mont.; I. L. Hathaway, Nebr.; B. L. Herrington, N. Y. (Cornell); W. J. Peterson, N. C.; W. E. Krauss, Ohio; J. R. Haag, Oreg.; R. A. Dutcher and N. B. Guerrant, Pa.; U. S. Ashworth and Margaret McGregor, Wash.; W. H. Peterson, Wis.; H. S. Willard, Wyo., and H. G. Wiseman and W. A. Turner, Bureau of Dairy Industry.

INTRODUCTION

A Nation-wide study to determine the seasonal variation in the vitamin A potency of milk and butter produced and marketed in different regions of the United States was begun in the spring of 1943 by the United States Department of Agriculture and 21 cooperating State agricultural experiment stations to answer certain questions raised by the Committee on Food and Nutrition of the National Research Council. The Committee (now the Food and Nutrition Board) wanted to be able to estimate how much vitamin A the people of the United States were actually getting from the butter they were consuming.

This publication brings together the results obtained up to and including August 1945, as reported by each of the cooperating States to the Technical Committee that was in charge of the study. The data bear mainly on three questions:

1. The vitamin A potency of the creamery butter produced in the various States.
2. The effect of commercial storage and handling on the vitamin A potency of butter.
3. The vitamin A potency of butter sold on retail markets in the United States.

It is not essential that the day-to-day consumption of vitamin A be the same as the recommended daily allowance. When more vitamin A is consumed than is required for immediate use, the excess is stored in the body and used later if the intake is deficient. Possibly under ordinary circumstances an adequate amount of vitamin A is obtained if the average daily intake from month to month approximates the recommended daily allowance. Therefore, effort has been made in this report to group the results from each State in such a way as to arrive at the average monthly vitamin A potency of the milk and butter produced.

Some of the State experiment stations have already published detailed accounts of their work and results (*1, 4, 5, 12, 14, 17, 20, 23, 24, 25*)[3] and others are expected to do the same. The purpose of this publication is to present a summary of the results now available on a Nation-wide basis in order to answer, entirely for practical purposes, the questions raised by the National Research Council. The report also includes a discussion of the reliability of the analytical methods used in determining the carotene and vitamin A content of the butter and also the procedures used in calculating the total vitamin A potency.

SUMMARY OF THE RESULTS

Results are available which indicate the vitamin A potency of the butter produced in creameries in 16 States. These States make 64 percent of the creamery butter produced in the United States, and their combined output undoubtedly may be considered representative of practically all the creamery butter produced in this country.

In all but two States there was a distinct difference between the vitamin A potency of butter produced under winter-feeding conditions

[3] Figures in parentheses refer to Literature Cited, p. 46.

and that produced under summer-feeding conditions. About 35 percent of all the creamery butter is "winter" butter and it has an average vitamin A potency of 10,500 or 11,200 International Units per pound, depending on the method of calculation. About 65 percent of all creamery butter is "summer" butter—produced by cows on pasture—and it has an average vitamin A potency of 17,000 or 18,000 I. U. per pound.

Careful consideration of all the results of this study indicates that the average vitamin A potency of the total output of creamery butter in the United States is approximately 15,000 I. U. per pound. Thus, when the people eat as much butter as they did immediately before the war (approximately 18 pounds annually per capita), butter furnishes about 740 I. U. of vitamin A per capita per day, or about 14.8 percent of the daily allowance (5,000 I. U.) recommended for normal adults by the Food and Nutrition Board of the National Research Council.

The effect of commercial storage and handling on the vitamin A potency of butter was studied by seven of the State experiment stations, and the results indicate that the effect of storage and handling is negligible.

Four State experiment stations investigated the vitamin A potency of butter sold on retail markets in their respective areas. They found no significant difference between the annual average vitamin A potency of the butter on the retail markets and that of the creamery butter produced in this country.

According to the results of this study, milk produced under winter-feeding conditions has an average vitamin A potency of approximately 1,120 I. U. per quart (4-percent-fat basis) and that produced under average summer-feeding conditions has a potency of about 1,820. The average potency of the total supply of milk which disappears annually for domestic consumption is 1,540 I. U. per quart. When the disappearance of milk for domestic consumption averages 1.052 quarts per capita per day, as it did in 1941, milk and milk products furnish the total population of this country slightly over 1,600 I. U. of vitamin A per capita per day, or nearly one-third of the recommended daily allowance.

The results of this study afford a practical demonstration of the fact, previously established by many investigators, that the vitamin A potency of milk and butter is dependent on the quantity of carotene in the diet of the cow. Since much of the summer milk has a vitamin A potency of from 2,500 to 3,000 I. U. per quart and some of the butter has well over 20,000 units per pound, it is apparent that much could be done to improve the average potency of the annual output by adopting better feeding practices in winter, and possibly in summer also.

PROCEDURES USED IN CONDUCTING THE STUDY

Method of Determining the Vitamin A Potency of the Butter

The vitamin A content of the butter was determined by using the antimony trichloride method, taking into account the color given by other materials in the butter which react with this reagent. The

procedure was a modification of the one used by Koehn and Sherman in analyzing cod-liver oil (*19*).

The carotene content of the butter was determined by washing the carotene solution in Skellysolve B either with 92-percent methyl alcohol or with 94-percent diacetone alcohol to separate the carotene from the other pigments in the butter, and then reading the carotene fraction either with a colorimeter or a spectrophotometer.

The above methods gave the results in micrograms of vitamin A and in micrograms of carotene, which taken together represent the total vitamin A potency of the butter. (See pp. 34–45 for further discussion of analytical methods.)

Method of Expressing the Results

The Technical Committee agreed that the cooperating laboratories should report both the amount of carotene and the amount of vitamin A itself, in micrograms, as determined in the butter. The Committee recognizes that for practical purposes it is desirable to express the total vitamin A value of the butter either in International Units or in United States Pharmacopoeia Units—the forms in which human and animal requirements are generally expressed—but it does not feel that it would be advisable at present to make general recommendations regarding methods of converting the micrograms of vitamin A into these units. For the purpose of this report, the Committee has decided to assume that all the pigment obtained in the "carotene" fraction by the chemical method was beta-carotene; and it has agreed that 0.6 microgram of the carotene and 0.25 microgram of the vitamin A shall each be taken as equal to 1 I. U. of vitamin A.

During the course of the survey different laboratories made numerous tests to determine how much vitamin A was lost in analyzing the butter. The tests were made with butter to which known quantities of crystalline vitamin A had been added. In the Bureau of Dairy Industry laboratory the average amount of vitamin A recovered with 20 samples of vitamin A alcohol was 93.0 percent, and with 6 samples of the vitamin A acetate it was 94.4 percent. The vitamin A results expressed in micrograms in this report are not corrected for this loss; the results in International Units are given without this correction and also as corrected for a recovery of 93 percent of the vitamin A, both values expressing within the limit of experimental error the potencies of butters that were assayed biologically. (See pp. 34–40 for further discussion of this subject.)

Method of Obtaining Butter Samples

The Technical Committee sent suggestions to each State for organizing the sampling of the butter. Most of the States divided their butter-producing sections into areas in which production conditions were more or less uniform and collected samples from representative creameries in each production area, usually at weekly or monthly intervals. The number of samples collected depended on the capacity of the State experiment station to do the analytical work. Fortunately, some of the largest butter-producing States participated

in this study. The extent of the sampling in these States leaves little doubt about the vitamin A potency of their product or of that produced in other States under similar conditions. Since each State is expected to publish its own results, only a brief account of the sampling phase will be included here in connection with the following discussion of the results by States and regions.

VITAMIN A POTENCY OF CREAMERY BUTTER BY STATES AND REGIONS

North Central States

Minnesota.—Creamery butter is produced in 86 of the 87 counties in Minnesota. On the basis of 1940 production figures, 10 sampling regions were established, each composed of a block of adjacent counties and each producing approximately 10 percent of the State's butter. Four creameries in each region agreed to submit samples at weekly intervals during 1943, and only 1 of the 40 failed to cooperate. All samples were 1-pound prints from the regular make (except the 10-pound tub received from each region each month for the storage studies which are presented later in this report). The samples were mailed unrefrigerated to the laboratory, the summer samples being packed in metal containers to avoid leakage.

Except for a transition period from winter feeding to pasture conditions, when all samples received were analyzed, analyses were made at biweekly intervals only—those for two creameries in each region being made on the odd-numbered weeks' samples and those for the other two on the even-numbered weeks' samples.

The roughage available in Minnesota during the winter of 1942–43 had been rather badly weathered at harvest. For this reason the project was extended through the first 6 months of 1944. For this extended portion of the project, samples were received from 1 creamery in each of the 10 regions at semimonthly intervals during January, February, March, and April, and at weekly intervals from May 15 to June 15, inclusive. All of these samples were analyzed, making a total of 1,145 samples analyzed. A complete report of the survey in Minnesota has been published (*17*). Table 1 shows the vitamin A potency of the butter.

Wisconsin.—The results of the work done at the Wisconsin Agricultural Experiment Station on the vitamin A potency of butter have been published (*4*, *5*).

Early in April 1942, 22 samples of butter (1-pound prints) made during the week of March 23, 1942, by creameries in southwestern Wisconsin, were analyzed. In July 1942, 20 samples of butter (15 from the same creameries, 5 from others in the same region) were analyzed.

The State was divided into 9 districts and samples from 70 different creameries were collected in September 1942, and other samples from 65 of these creameries were collected in January 1943. The results on the vitamin A potency of these creamery butters are summarized in table 2.

TABLE 1.—*Vitamin A potency of creamery butter produced in Minnesota*

Production period and number of samples	Carotene per gram of butter	Vitamin A per gram of butter	Potency per pound	
			Not corrected for loss in analysis	Corrected for loss of vitamin A in analysis [1]
1943 (1,019 samples):	*µg.*	*µg.*	*I. U.*	*I. U.*
Jan. 1–28	1.89	4.66	9,892	10,540
Jan. 29–Feb. 25	1.82	4.59	9,712	10,455
Feb. 26–Mar. 25	1.82	4.32	9,222	9,826
Mar. 26–Apr. 22	1.53	4.84	9,947	10,602
Apr. 23–May 6	1.88	5.52	11,446	12,160
May 7–20	3.33	6.85	14,960	15,835
May 21–June 17	6.09	7.42	18,083	18,910
June 18–July 15	5.53	6.82	16,569	17,580
July 16–Aug. 12	4.82	7.14	16,613	17,820
Aug. 13–Sept. 9	5.18	7.98	18,412	19,633
Sept. 10–Oct. 7	5.66	7.96	18,738	19,937
Oct. 8–Nov. 4	5.51	7.59	17,952	19,054
Nov. 5–Dec. 2	3.94	6.01	13,895	14,668
Dec. 3–31	2.74	5.28	11,661	12,325
Mean, Jan. 1–June 17 [2]			11,968	
Mean, Jan. 1–Mar. 25 [2]			9,601	
Mean, December–April [2] [3]			10,046	10,700
Mean, May–November [2] [4]			16,809	17,790
Mean, annual [2]			13,958	14,793
1944 (126 samples):				
January	2.01	5.19	10,946	
February	1.76	3.97	8,540	
March	1.74	4.22	8,981	
April	1.70	4.60	9,640	
May 1–15	3.11	7.68	16,301	
May 16–22	4.45	7.61	17,198	
May 23–31	6.00	7.45	18,070	
June 1–8	5.75	7.30	17,609	
June 9–15	5.41	7.08	16,951	
Mean [2]			11,974	
Mean, January, February, March [2]			9,495	

[1] Using a recovery of 93 percent for the vitamin A in the butter, the same as found for crystalline vitamin A when added to butter before analysis.
[2] Weighted for period productions.
[3] Taken as "winter" months, representing 42.3 percent of the 1943 annual output.
[4] Taken as "summer" months, representing 57.7 percent of the 1943 annual output.

TABLE 2.—*Vitamin A potency of creamery butter produced in Wisconsin*

Number of samples and date analyzed or collected	Carotene per gram in butterfat	Vitamin A per gram in butterfat	Potency of butter per pound	
			Not corrected for loss in analysis	Corrected for loss of vitamin A in analysis
	µg.	*µg.*	*I. U.*	*I. U.*
22 samples, March 1942	2.16	5.61	9,568	10,185
65 samples, January 1943	2.97	5.89	10,488	11,141
20 samples, July 1942	7.43	8.96	17,738	18,731
70 samples, September 1942	7.66	9.06	18,032	19,034
Average of winter samples [1]	2.56	5.75	10,028	10,663
Average of summer samples [2]	7.54	9.01	17,885	18,883
Mean for annual output [3]	5.82	7.88	15,167	16,039

[1] Taken to represent the average potency of winter butter—i. e. butter produced in December to April, inclusive, which was 34.6 percent of the State's annual output.
[2] Taken to represent the average potency of summer butter—i. e. butter produced in May to November, inclusive, which was 65.4 percent of the State's annual output.
[3] Weighted for seasonal productions.

IOWA.—This State was divided into 7 sampling areas, in each of which the conditions of production were reasonably uniform. It was planned to sample the butter in 60 creameries, distributed in proportion to the production of the area relative to the State output, and to take 1 sample per month from each creamery. The survey was continued for 24 months; 1,119 samples were analyzed. The results for each year and the combined means for 2 years are shown in table 3.

TABLE 3.—*Vitamin A potency of the creamery butter produced in Iowa*

Production period	Carotene per gram of butter [1]	Vitamin A per gram of butter [1]	Potency per pound	
			Not corrected for loss in analysis	Corrected for loss of vitamin A in analysis
1943	*µg.*	*µg.*	*I. U.*	*I. U.*
July	8.62	7.26	19,710	21,190
August	6.02	8.47	19,940	21,440
September	6.06	8.23	19,530	21,000
October	5.13	7.35	17,230	18,520
November	4.96	6.75	16,010	17,210
December	2.72	6.16	13,240	14,230
1944				
January	1.57	5.10	10,450	11,230
February	1.41	5.13	10,390	11,180
March	1.19	4.43	8,940	9,610
April	1.18	4.65	9,340	10,040
May	3.11	6.50	14,160	15,220
June	5.02	6.58	15,750	16,930
July	4.05	6.75	15,320	16,470
August	4.26	7.28	16,440	17,670
September	4.81	7.62	17,480	18,800
October	5.60	7.69	18,200	19,570
November	5.03	6.45	15,520	16,680
December	3.63	5.02	11,870	12,770
1945				
January	2.06	4.04	8,890	9,560
February	1.49	3.67	7,790	8,380
March	1.28	3.73	7,740	8,320
April	2.16	4.81	10,370	11,150
May	4.87	5.84	14,290	15,370
June	6.13	6.31	16,100	17,320
Mean, winter (December 1943–April 1944) [2]			10,360	11,040
Mean, summer (July–November 1943 and June 1944) [2]			17,490	18,500
Mean, annual (July 1943–June 1944) [2]			14,890	15,780
Mean, winter (December 1944–April 1945) [2]			9,280	9,860
Mean, summer (July–November 1944 and June 1945) [2]			16,040	16,970
Mean, annual (July 1944–June 1945) [2]			13,550	14,350
Mean, winter (both years) [2]			9,830	10,470
Mean, summer (both years) [2]			16,820	17,760
Mean, annual (both years) [2]			14,250	15,090

[1] Weighted for area productions.
[2] Weighted for monthly, seasonal, and yearly productions.

KANSAS.—Samples of butter were obtained from seven creameries at approximately monthly intervals (*23*). The creameries were selected on the basis of their geographic location and method of operation. Six of the plants obtain their cream from local sources, whereas the other plant is a large centralizer creamery which obtains its cream from widely scattered sources. These seven creameries represented 20.2 percent of the estimated butter production in Kansas in 1944. The results for 18 months (126 samples) are shown in table 4.

TABLE 4.—*Vitamin A potency of creamery butter produced in Kansas* [1]

Production period	Carotene per gram of butter	Vitamin A per gram of butter	Potency per pound: Not corrected for loss in analysis	Potency per pound: Corrected for loss of vitamin A in analysis
1943	*µg.*	*µg.*	*I. U.*	*I. U.*
July	5.61	5.92	15,000	15,800
August	5.32	4.88	12,900	13,550
September	3.62	6.33	14,200	15,100
October	3.53	5.44	12,550	13,300
November	3.28	4.94	11,500	12,100
December	2.42	4.69	10,350	11,000
1944				
January	1.86	4.07	8.800	9,350
February	1.63	3.95	8,400	8,950
March	1.74	5.15	10,700	11,400
April	2.65	6.07	13,050	13,850
May	5.02	8.15	18,600	19,750
June	5.25	8.2	18,850	20,000
July	5.03	6.19	15,050	15,950
August	4.71	6.25	14,900	15,800
September	4.70	6.67	15,700	16,600
October	4.88	6.89	16,200	17,150
November	5.51	6.69	16,300	17,250
December	3.32	4.44	10,600	11,300
Mean for year, July 1943–June 1944 [2]			13,370	14,170
Mean for year 1944 [2]			14,250	15,100
Mean for all winter months (December–April) [2]			10,414	11,046
Mean for all summer months (May–November) [2]			16,020	16,972
Mean for year using all monthly means [2]			13,915	14,743

[1] Each monthly value represents the mean for 7 creameries weighted according to their monthly pro ductions.

[2] Mean of the monthly means weighted for monthly productions. When samples were taken in the same month in both 1943 and 1944 the average production and weighted mean of the potencies for the 2 months are used. Mean winter production was 37.6 percent and mean summer production 62.4 percent of annual production, using all months and the averages for the months in which samples were taken in both 1943 and 1944.

NEBRASKA.—The samples of butter were obtained at monthly intervals from sampling centers suggested by the Butter Institute. They were as follows: Southeastern, Beatrice Creamery Company, Lincoln; Central, Fairmont Creamery Company, Grand Island; Western, Fairmont Creamery Company, Alliance; Northeastern, Swift & Co., Hastings.

A total of about 88 samples were analyzed. The results are shown in table 5.

OHIO.—Twelve creameries were selected as representative of the State's output of creamery butter. A 1-pound print of butter was collected from each creamery either monthly or bimonthly. The sampling was continued for 2 years, 18 sets of samples being taken. The station has published a brief report of the work (*20*) and the results are summarized in table 6.

SUMMARY OF NORTH CENTRAL STATES.—The creamery butter produced in the North Central States is about 77.1 percent of the total production in the United States; the six North Central States that participated in this phase of the study produce 54.4 percent of the total national output. It seems likely that the results obtained in these six States represent closely the vitamin A potency of the North

TABLE 5.—*Vitamin A potency of creamery butter produced in Nebraska*

Production period	Carotene per gram of butter	Vitamin A per gram of butter	Potency per pound: Not corrected for loss in analysis [1]	Potency per pound: Corrected for loss of vitamin A in analysis [1]
1943 and 1944	*μg.*	*μg.*	*I. U.*	*I. U.*
October	6.52	7.58	18,700	19,734
November	6.16	6.69	16,800	17,713
December	4.37	6.60	15,295	16,200
1944 and 1945				
January	3.39	5.30	12,188	12,911
February	2.24	5.16	12,420	13,129
March	2.09	5.12	10,880	11,576
April	2.07	5.07	10,773	11,467
May	3.88	7.86	17,220	18,295
June	7.82	10.04	24,143	25,517
July	8.68	8.56	22,107	23,290
1944				
August	7.52	9.02	22,065	23,302
September	6.20	8.87	20,795	22,008
Mean, winter (December–April)[2] [3]			12,160	12,890
Mean, summer (May–November)[2] [4]			20,670	21,850
Mean, annual [2]			17,470	18,480

[1] Weighted for area productions and for productions for the same month for 2 years.
[2] Weighted using average of monthly productions for 2 years.
[3] Production for December to April, inclusive, was 37.5 percent of the annual output.
[4] Production for May to November, inclusive, was 62.5 percent of the annual output.

TABLE 6.—*Vitamin A potency of creamery butter produced in Ohio*

Production period	Carotene per gram of butter	Vitamin A per gram of butter	Potency per pound: Not corrected for loss in analysis	Potency per pound: Corrected for loss of vitamin A in analysis
1943	*μg.*	*μg.*	*I. U.*	*I. U.*
March	2.46	3.53	8,149	8,762
May	6.18	4.93	13,629	14,301
July	6.35	5.68	15,118	15,899
September	6.88	5.06	14,396	15,068
November	5.25	4.99	13,034	13,724
1944				
January	3.37	3.85	9,543	10,070
March	2.62	3.86	8,994	9,520
April	3.10	4.37	10,238	10,882
May	7.18	5.65	15,695	16,458
June	7.17	5.29	15,032	15,785
July	6.00	5.83	15,127	15,926
August	5.74	5.62	14,551	15,313
September	6.77	5.99	15,999	16,816
November	7.09	5.26	14,918	15,645
December	5.84	5.11	13,697	14,387
1945				
January	3.12	4.06	9,734	10,297
February	2.61	3.77	8,821	9,330
March	2.46	3.99	9,107	9,652
1943, 1944, and 1945 combined: [1]				
Mean, winter months (December–April)			10,250	10,830
Mean, summer months (May–November)			14,820	15,560
Mean, annual			13,407	14,107

[1] The potency for a given month is weighted for its production in the successive years. The average monthly productions for the successive years are averaged and the average used in weighting to get the winter and summer means. The annual mean is for 2 years (March 1943 to February 1945) weighted for seasonal productions.

762762°—47——2

Central States as a whole and, therefore, the potency of about 77 percent of the creamery butter produced in the United States. The results are summarized in table 7.

TABLE 7.—*Summary of results on the vitamin A potency of creamery butter produced in 6 North Central States*

State	Annual butter production (Average 1942–43)	Winter butter production [1]	Summer butter production [1]	Mean potency per pound of butter					
				Winter (December–April)		Summer (May–November)		Annual	
				Not corrected for loss in analysis	Corrected for loss of vitamin A in analysis	Not corrected for loss in analysis	Corrected for loss of vitamin A in analysis	Not corrected for loss in analysis	Corrected for loss of vitamin A in analysis
	Million pounds	*Percent*	*Percent*	*I. U.*	*I. U.*	*I. U.*	*I. U.*	*I. U.*	*I. U.*
Minn	304.4	42.3	57.7	10,046	10,700	16,809	17,790	13,958	14,793
Wis	151.2	34.6	65.4	10,028	10,663	17,885	18,884	15,167	16,039
Ohio	69.5	30.9	69.1	10,250	10,830	14,820	15,560	13,407	14,107
Iowa	241.0	36.7	63.3	9,830	10,470	16,820	17,760	14,250	15,090
Nebr	93.3	37.5	62.5	12,160	12,890	20,670	21,850	17,470	18,480
Kans	74.7	37.6	62.4	10,414	11,046	16,020	16,972	13,915	14,743
Total or mean [2]	934.1	37.9	62.1	10,240	10,880	17,150	18,120	14,540	15,390
Standard error [3]				±119	±124	±254	±272	±198	±209
Coefficient of variation [3]				2.9	2.8	3.6	3.7	3.3	3.3

[1] Percentages are based on annual productions during the sampling periods.
[2] Weighted for production in participating States.
[3] The standard error is calculated on the variations of the individual State means from the mean of these individual means.

NOTE.—Total output for the North Central States was 1,324.9 million pounds or 77.1 percent of the national output. The production in the participating States was 70.5 percent of that of the group or 54.4 percent of the national output.

It will be noted (table 7) that the potency of the winter butter produced in the creameries of the North Central States is quite uniform, the mean and its standard error being either 10,240±119 or 10,880±124 I. U. per pound (i. e., between 10,000 and 11,000 I. U. per pound) depending on whether or not the results are corrected for loss of vitamin A in analysis.

The potency of the butter produced during the summer varied considerably more than that produced during the winter, the mean and its standard error for the summer butter being 17,150±254 or 18,120±272 (i. e., about 17,000 to 18,000 I. U.). The coefficient of variation is obviously greater than that for the winter product. The average and standard error for the annual potency of the butter produced in these States was 14,540±198 or 15,390±209 I. U. per pound.

Variations in summer butter may occur from season to season in the same State or between different months of the same season. Such differences result from variations in the condition of the pasture, due to the effect of differences in rainfall, etc. The Technical Committee would have to know more about the weather and pasture conditions at the time of sampling, and about the relation of these conditions to the average summer conditions, in order to determine whether the differences observed from State to State are at all general or character-

istic. The seasonal uniformity of the vitamin A potency of the winter and summer products from State to State is much more impressive than the differences in potency.

South Atlantic and South Central States

North Carolina.—Ten creameries were selected from which to obtain samples. They represent 90 percent of all the creamery butter produced in the State. Seven of the creameries were in the Piedmont section and three were in the Mountain section, and this is in proportion to the production of creamery butter in these areas. The results are shown in table 8.

Table 8.—*Vitamin A potency of creamery butter produced in North Carolina*

Production period	Carotene per gram of butter	Vitamin A per gram of butter	Potency per pound	
			Not corrected for loss in analysis	Corrected for loss of vitamin A in analysis
	µg.	*µg.*	*I. U.*	*I. U.*
August 1943 (10 creameries)	5.9	6.6	16,447	17,350
June 1944 (8 creameries)	8.2	5.1	15,467	16,167
November 1943 (10 creameries)	4.9	4.0	10,972	11,517
February 1944 (9 creameries)	3.5	3.6	9,184	9,674
Mean, December–March			9,184	9,674
Mean, April–November [1]			15,476	16,253
Mean, annual [2]			13,619	14,312

[1] The average of August 1943 and June 1944 is taken as representative of the April–October output, and this average is weighted with that of November 1943 to get the summer mean (i. e. mean for April–November). The average for February is taken to represent the winter product.
[2] These figures taken as seasonal means were weighted according to the 1943 State production—29.5 percent for December–March; 70.5 percent for April–November.

Mississippi.—The butter was obtained the first of each month from three creameries representing probably 60 percent of the butter made in Mississippi. The results are shown in table 9.

At creamery No. 1 (table 9) the butter was made from cream separated on the farm and delivered sweet to the plant, where it was pasteurized at 155° F. for 30 minutes.

At creamery No. 2 (table 9) the butter was made from cream separated on the farm and delivered sour to the plant, where it was neutralized and pasteurized at 185° to 200° F.

At creamery No. 3 (table 9) the butter was made from sweet cream separated at the plant and pasteurized at 155° F. for 30 minutes.

The results on the different types of butter (table 9) were weighted according to the information furnished by the Mississippi station to the effect that 25 percent of Mississippi butter is made from sweet cream separated on the farm, 50 percent from cream separated on the farm and delivered sour to the creamery, and 25 percent from milk separated at the creamery, except that during November, December, January, February, and March, the amount of cream delivered sour to the plant probably drops to around 40 percent of the total.

Regarding the difference in vitamin A potency between butter made

TABLE 9.—*Vitamin A potency of creamery butter produced in Mississippi*

Production period	Carotene per gram of butter	Vitamin A per gram of butter	Potency per pound: Not corrected for loss in analysis	Potency per pound: Corrected for loss of vitamin A in analysis
Creamery No. 1:	*µg.*	*µg.*	*I. U.*	*I. U.*
March 1943	3.0	5.03	12,623	13,420
March 1944	2.4	6.63		
April 1943	4.8	6.63	16,723	17,673
April 1944	6.0	7.29		
May 1944	8.1	7.54	19,813	20,844
June 1943	9.6	5.21	17,042	17,843
June 1944	7.3	6.52		
July 1943	7.3	5.99	16,392	17,213
August 1943	7.8	6.46	17,640	18,520
September 1943	7.8	5.94	16,678	17,490
October 1943	7.0	6.56	17,215	18,108
November 1943	6.1	5.82	15,184	15,983
December 1943	3.8	5.53	12,920	13,672
January 1944	3.8	5.32	12,528	13,255
February 1944	3.2	5.00	11,494	12,175
Mean, December–March [1]			12,419	13,161
Mean, April–November [1]			17,401	18,292
Mean, annual [1 2]			16,267	17,124
Creamery No. 2:				
March 1943	1.7	3.49	7,534	8,179
March 1944	1.2	3.77		
April 1943	2.7	4.76	13,645	14,474
April 1944	4.3	7.35		
May 1944	6.6	6.99	17,685	18,638
June 1943	9.5	4.70	16,672	17,442
June 1944	7.5	6.58		
July 1943	7.1	6.64	17,436	18,339
August 1943	7.2	6.99	18,130	19,088
September 1943	7.8	5.85	16,525	17,322
October 1943	5.2	6.62	15,960	16,864
November 1943	5.3	5.24	13,528	14,240
December 1943	3.6	5.11	12,002	12,696
January 1944	2.6	5.06	11,153	11,843
February 1944	2.2	4.28	9,442	10,028
Mean, December–March [1]			9,994	10,649
Mean, April–November [1]			16,370	17,234
Mean, annual [1 2]			14,918	15,735
Creamery No. 3:				
March 1943	2.9	4.50	10,500	11,174
March 1944	1.2	5.36		
April 1943	5.1	6.68	16,421	17,374
April 1944	4.8	7.29		
May 1944	7.4	7.19	18,652	19,632
June 1943	10.6	5.20	17,869	18,696
June 1944	7.6	6.89		
August 1943	8.4	6.91	18,892	19,838
September 1943	9.2	6.16	18,153	18,992
October 1943	6.7	6.69	17,208	18,122
November 1943	5.7	5.39	14,095	14,830
December 1943	4.1	5.44	12,978	13,723
January 1944	4.9	5.71	14,072	14,848
February 1944	2.1	4.76	10,232	10,881
Mean, December–March [1]			11,977	12,689
Mean, April–November [1]			17,499	18,402
Mean, annual [1 2]			16,120	16,971
Mean for State output: [3]				
Winter (December–March)			11,316	12,014
Summer (April–November)			16,924	17,868
Annual [2]			15,645	16,533

[1] Weighted for monthly production of State.
[2] The winter output of creamery butter was estimated at 22.8 percent and the summer output at 77.2 percent of the annual output of the State.
[3] Weighted for monthly production and for proportions of sweet and sour cream butter.

from sweet and sour creams, Professor F. H. Herzer of the Mississippi station reported:

Our work indicated that cream delivered sour produced butter which had about 15 percent on the average lower vitamin A content than the butter from the sweet cream. This fact seemed so significant I felt it wise to determine what effect holding cream under farm conditions for 10 days, as is commonly practiced by some small producers, would have on the butter made from this cream. Some four or five runs have been made on the same cream (1) on butter made from cream immediately after separation; (2) the same cream pasteurized and churned; (3) the same cream held for 3 days, pasteurized, neutralized, and churned; and the same operation for two more successive 3-day periods. To date we do not have any significant trend to establish the fact that holding cream on the farm for considerable length of time has any effect on the lowering of the vitamin A content. Just why the sour cream butter consistently ran lower I am now at a loss to state.

LOUISIANA.—Samples were taken from two creameries—one in the northern part and one in the southern part of the State—which represented about two-thirds of the creamery butter produced in the State (*12*). The data given in table 10 are the means of the results from these two creameries for 2 years.

TABLE 10.—*Vitamin A potency of creamery butter produced in Louisiana*[1]

Production period	Carotene per gram of butter	Vitamin A per gram of butter	Potency per pound: Not corrected for loss in analysis	Potency per pound: Corrected for loss of vitamin A in analysis
1943 and 1944	*µg.*	*µg.*	*I. U.*	*I. U.*
September	5.99	7.18	17,570	18,550
October	5.67	7.88	18,600	19,680
November	4.37	5.00	12,385	13,070
December	3.93	4.77	11,636	12,289
1944 and 1945				
January	3.25	3.68	9,140	9,640
February	3.18	5.24	11,922	12,640
March	5.20	6.18	15,160	16,012
April	6.96	6.33	16,770	17,637
May	7.72	6.23	17,151	18,005
June (1945 only)	6.77	4.90	14,021	14,691
July	5.04	6.52	15,644	16,534
August (1944 only)	5.32	6.01	14,940	15,758
Mean, November–February			11,361	11,990
Mean, March–October			16,220	17,090
Mean, annual			14,970	15,780

[1] The monthly results from the two creameries were weighted for the monthly productions of these creameries to obtain each month's mean potency. This was taken as representing the potency for the creamery butter produced in the State for that month. These mean monthly potencies were weighted for the State monthly productions for successive years in getting the mean monthly potencies given; and the averages of the monthly productions for these years were used in getting the summer, winter, and annual potencies.

SUMMARY OF SOUTH ATLANTIC AND SOUTH CENTRAL STATES.—A summary of the results on the vitamin A potency of butter produced in the South Atlantic and South Central States that participated in this study is given in table 11.

ROCKY MOUNTAIN AND PACIFIC COAST STATES

WASHINGTON.—A report showing the carotene and vitamin A content of creamery butter produced in Washington has been published (*1*). This State was divided into three areas: (1) West Coast,

TABLE 11.—*Summary of results on the vitamin A potency of the butter produced in the South Atlantic and South Central States* [1]

State	Annual butter production (average 1942–43)	Winter butter production [2]	Summer butter production [2]	Mean potency per pound of butter: Winter butter, Not corrected for loss in analysis	Winter butter, Corrected for loss of vitamin A in analysis	Summer butter, Not corrected for loss in analysis	Summer butter, Corrected for loss of vitamin A in analysis	Annual production, Not corrected for loss in analysis	Annual production, Corrected for loss of vitamin A in analysis
	1,000 pounds	*Percent*	*Percent*	*I. U.*	*I. U.*	*I. U.*	*I. U.*	*I. U.*	*I. U.*
North Carolina	1,812	29.5	70.5	9,184	9,674	15,476	16,253	13,619	14,312
Louisiana	770	30.2	69.8	11,361	11,990	16,220	17,090	14,970	15,780
Mississippi	3,990	22.8	77.2	11,316	12,014	16,924	17,868	15,645	16,533
Total or mean [3]	6,572	25.5	74.5	10,640	11,270	16,480	17,360	14,990	15,807

[1] The annual butter production in the 13 States in this group (North Carolina, Louisiana, Mississippi, Alabama, Texas, Virginia, South Carolina, Georgia, Florida, Tennessee, Kentucky, Arkansas, and Oklahoma) was 151,134,000 pounds in 1942–43. The production in the participating States in this group was about 4.3 percent of that of the group.
[2] Based on annual production during sampling periods.
[3] Weighted for production in participating States.

(2) Yakima Valley, and (3) the East Side dry-farming area. These areas represent 54 percent, 21 percent, and 25 percent, respectively, of the State's creamery butter production. Samples were taken at six, three, and three creameries, respectively, in these areas. The samples were collected by State inspectors and mailed to the laboratory. Two samples per month were taken from each creamery. The results are summarized in table 12.

TABLE 12.—*Vitamin A potency of creamery butter produced in Washington* [1]

Production period	Carotene per gram of butter	Vitamin A per gram of butter	Potency per pound: Not corrected for loss in analysis	Potency per pound: Corrected for loss of vitamin A in analysis
1943	*µg.*	*µg.*	*I. U.*	*I. U.*
May–June	9.72	8.28	21,330	22,400
August	6.73	8.22	18,970	20,000
November	6.33	7.00	16,610	17,500
1944				
February	3.79	5.08	11,450	12,100
May–June	9.50	7.23	19,410	20,300
August	7.60	6.13	16,100	16,900
November	7.64	6.18	16,210	17,000
1945				
February	3.88	4.93	11,260	11,900
Mean, winter [2]			11,355	12,000
Mean, summer [3]			18,640	19,580
Mean, annual [4]			17,458	18,344

[1] The results in the table are State means weighted for area productions, using 1943 production figures.
[2] The February 1944 and February 1945 results are taken to represent winter (December, January, February) butter.
[3] The mean of the May–June, August, and November potencies, weighted for the productions for these months in 1943 and 1944, are taken to represent summer butter.
[4] The seasonal means weighted for seasonal productions for the 2 years, March 1943–February 1945, both months inclusive, i. e. winter 16.28 percent, summer 83.72 percent of annual production.

Oregon.—Samples of butter were taken at irregular intervals from 10 creameries—3 representing the Valley area, 2 representing the Coastal area, and 5 representing the Central, Eastern, and Southern areas. These areas represent the "predominant feeding practices" in the State. The results are shown in table 13. In addition, the Oregon station conducted some experiments with "experimental rations." These results are shown in table 14. These latter results indicate the changes that may be effected in the vitamin A potency of butter through changes in feeding practices.

Table 13.—*Vitamin A potency of creamery butter produced in Oregon*

Predominant roughage fed [1]	Carotene per gram of butter [2]	Vitamin A per gram of butter [2]	Potency per pound	
			Not corrected for loss in analysis [2]	Corrected for loss of vitamin A in analysis [2]
	µg.	*µg.*	*I. U.*	*I. U.*
Pasture:				
April 1945 (1)	6.95	6.74	17,488	18,408
May 1944, 1945 (3)	10.08	6.50	19,430	20,320
June 1943, 1944, 1945 (12)	9.07	6.03	17,810	18,640
September 1944 (1)	4.70	5.51	13,571	14,316
October 1944 (1)	6.16	5.41	14,485	15,231
Mixed hay, alfalfa; no pasture:				
November 1943 (1)	5.60	4.09	11,663	12,221
December 1943, 1944 (3)	4.94	4.98	12,781	13,451
January 1945 (1)	5.18	5.01	13,019	13,705
February 1944, 1945 (8)	3.44	5.15	11,955	12,663
Mean potency:				
Pasture [3]			17,700	18,560
No pasture [3]			12,340	13,000
Annual [4]			15,740	16,520

[1] Figures in parentheses indicate the number of samples included in the average.
[2] Straight unweighted averages of individual samplings without regard to area productions or variations in production during the same month in successive years.
[3] Monthly averages weighted for the average of the monthly productions for 1943 and 1944.
[4] Using the mean for the "pasture" product to represent summer butter—i. e. butter produced in April–October (69.1 percent of annual output); and the no "pasture" product to represent winter butter—i. e. butter produced in November–March or 30.9 percent of the annual output.

Table 14.—*Carotene and vitamin A contents of butter produced on experimental rations in Oregon*

Ration and sampling date	Carotene per gram of butter	Vitamin A per gram of butter	Potency per pound	
			Not corrected for loss in analysis	Corrected for loss of vitamin A in analysis
Bleached hay: 2 p. p. m. crude carotene:	*µg.*	*µg.*	*I. U.*	*I. U.*
Nov. 12, 1943	0.97	2.08	4,512	4,798
U. S. No. 1 alfalfa hay:				
Nov. 12, 1943	3.08	7.11	15,240	16,216
Poor hay, some silage:				
Sept. 26, 1944	3.18	8.04	17,005	18,108
Oct. 21, 1944	4.21	9.46	20,364	21,657
Dec. 12, 1944	2.69	6.43	13,708	14,585
Jan. 1, 1945	1.94	5.72	11,855	12,633
3 p. p. m. crude carotene hay:				
Apr. 26, 1945	.68	3.50	6,869	7,345
Apr. 26, 1945	1.02	2.59	5,475	5,834
May 15, 1945	.90	4.78	9,360	10,010
Pasture 18–32 days:				
May 15, 1945	4.67	10.00	21,690	23,050
June 16, 1945	4.67	8.94	19,766	20,985
June 16, 1945	7.91	6.06	16,987	17,810

CALIFORNIA.—The State was divided into eight representative areas similar in climate, pasturage, and general dairy practices (*24*). Two or three creameries were selected in each area, which were believed to represent the output of the area. One-pound (sometimes 2-pound) samples were obtained, together with churn reports, from each creamery monthly. The butter analyzed represented almost 50 percent of the total butter production of the State.

The data shown in table 15 give the vitamin A potency of the creamery butter for March 1943 to February 1944, both inclusive.

TABLE 15.—*Vitamin A potency of creamery butter produced in California*

Production period	Carotene per gram of butter	Vitamin A per gram of butter	Potency per pound	
			Not corrected for loss in analysis	Corrected for loss of vitamin A in analysis
1943	μg.	μg.	I. U.	I. U.
Mean, March, April, May [1]	8.52	7.17	19,468	20,449
Mean, June, July, August [1]	6.45	7.02	17,628	18,588
Mean, September, October, November [1]	6.62	6.43	16,686	17,566
Mean, December 1943 and January, February 1944 [1]	5.25	5.75	14,414	15,200
Mean, March–November (summer) [2]			18,200	19,140
Mean, December–February (winter) [2]			14,414	15,200
Mean, annual [2]	6.94	6.69	17,420	18,330

[1] Weighted for area and monthly productions.
[2] Weighted for seasonal productions, using March–November for the summer months and December–April for the winter months with productions of 79.4 percent and 20.6 percent of the annual output, respectively.

IDAHO.—Semimonthly samples were taken of the butter produced in six creameries, representing 75 percent of the creamery butter produced in the State (*25*). The vitamin A potency of the butter is shown in table 16.

TABLE 16.—*Vitamin A potency of creamery butter produced in Idaho*

Production period	Carotene per gram of butter	Vitamin A per gram of butter	Potency per pound [1]	
			Not corrected for loss in analysis	Corrected for loss of vitamin A in analysis
1943	μg.	μg.	I. U.	I. U.
August	7.05	6.68	17,463	18,376
September	7.86	6.55	17,840	18,737
October	7.96	6.40	17,645	18,523
November	6.36	5.47	14,744	15,495
December	4.63	5.16	12,870	13,575
1944				
January	4.25	5.05	12,383	13,071
February	3.97	5.03	12,139	12,826
March	3.76	4.85	11,652	12,317
April	5.28	5.45	13,890	14,637
May	8.54	5.94	17,246	18,060
June	9.05	6.29	18,267	19,132
July	7.63	6.44	17,468	18,351
Mean, winter, December–April [2]			12,621	13,312
Mean, summer, May–November [2]			17,338	18,197
Mean, annual [2]			15,587	16,384

[1] Monthly averages unweighted for area production.
[2] Weighted for monthly productions; winter production 37.1 percent; summer 62.9 percent.

MONTANA.—This State was divided into five areas—two of them were typical irrigated areas; in the other three "dry-land" farming prevailed. Three times a year a butter sample (two 1-pound prints) was obtained from a creamery in each of these five areas. The first samples obtained were churned in the early part of April 1943, and represented "late winter" feeding conditions; the second samples were churned during the middle part of June 1943, and represented green grass pasture—i.e., spring-feeding conditions; and the third samples were churned during the middle part of October 1943, and represented dry pasture forage in "dry-land" areas, and some green feeds of greater maturity in the irrigated sections—i.e., fall conditions. In the irrigated areas, high-grade alfalfa hay was fed during the fall, winter, and early spring. In the unirrigated areas the pasturage in the late summer and early fall was dry, the cows were turned on stubble and cornstalk fields after the crops were harvested, and there was then very little green pasture feed available.

The kind, but not the quality, of hay fed in the unirrigated regions was given in the report received from the Montana laboratory. There was nothing in the description of winter feeding in the unirrigated areas to lead one to suspect that the butter produced in them would be unusually high in vitamin A content. The carotene in the butterfat from these areas in the winter was 1.59 to 2.45 micrograms per gram, which also would not indicate that the cows were receiving a ration unusually rich in carotene; and yet the vitamin A reported in the butter from these three areas varied from 8.82 to 13.34 micrograms per gram, and the potency of the butters from these different areas was reported at from 15,504 to 22,228 I. U. per pound, corrected for loss of vitamin A in analysis. The vitamin A found in the referee samples of butter analyzed by the Montana laboratory agreed very well with the average for all of the laboratories reporting; and it does not seem that the very high results obtained for winter butter in these unirrigated areas could very well be due to error in the calibration of the Evelyn machine that was used for the determination of the vitamin A. These results require further attention before their significance can be determined.

A summary of the Montana results is given in table 17.

TABLE 17.—*Vitamin A potency of creamery butter produced in Montana*

Production period	Carotene per gram of butter	Vitamin A per gram of butter	Potency per pound	
			Not corrected for loss in analysis	Corrected for loss of vitamin A in analysis
	µg.	*µg.*	*I. U.*	*I. U.*
Mean, late spring [1]	8.40	10.85	20,330	21,530
Mean, fall [1]	5.13	9.82	17,550	18,636
Mean, late winter [1]	5.14	12.52	21,552	22,907
Mean, annual [2]			19,801	21,014

[1] Weighted for area production.
[2] Weighted for area and seasonal production, on basis of figures supplied by the Montana laboratory. Fall production (29.7 percent) was taken as the season of minimum production.

762762°—47——3

WYOMING.—During 1944 and 1945, 7 creameries sent in 81 samples of creamery butter for analysis. These creameries represented areas of diverse altitude and different types of farms and ranches. In all cases alfalfa hay made up the sole roughage in winter, except in the southeastern part of the State where native hays made up the sole roughage. The summer forage consisted chiefly of native grass ranges. The altitude of the areas varied from 4,000 to 7,000 feet. The creameries represented the North Central area, east and west of the Big Horn mountains; the extreme west, south of the Tetons; the high valleys between the Medicine Bow and Laramie ranges. No appreciable difference was noted in the vitamin A potency of the butters from these several areas. These results are shown in table 18.

TABLE 18.—*Vitamin A potency of creamery butter produced in Wyoming*

Production period	Carotene per gram of butter	Vitamin A per gram of butter	Potency per pound	
			Not corrected for loss in analysis	Corrected for loss of vitamin A in analysis
1944	*µg.*	*µg.*	*I. U.*	*I. U.*
January	2.04	4.20	9,162	9,734
1944–45				
February	2.54	3.88	8,960	9,490
March	2.38	3.84	8,769	9,294
April	2.22	3.81	8,590	9,108
May	2.73	6.13	13,184	14,024
June	3.77	5.67	13,136	13,916
July	5.09	6.21	15,119	15,869
1945				
August	3.35	4.59	10,863	11,493
1943–44				
September	3.42	5.32	12,240	12,966
October	3.61	5.38	12,494	13,229
1944				
November	3.88	4.85	11,737	12,403
1943				
December	2.39	5.05	10,972	11,667
Mean, December–April [1]			9,170	9,750
Mean, May–November [1]			12,870	13,600
Mean, annual [1]			11,586	12,263

[1] Weighted for 1944 production as given in report from Wyoming—winter (December–April) 34.8 percent, summer 65.2 percent.

NOTE.—With 2 referee samples of butter in which the vitamin A was determined in 14 other laboratories and the carotene in 16 other laboratories (see table 31) the Wyoming results were lower than the averages of the other laboratories. With one sample of Wyoming butter analyzed for carotene and vitamin A in the Wyoming and one other laboratory there were similar differences. If these differences were allowed for, the mean annual average potency of Wyoming butter would be close to the national average.

ARIZONA.—Samples of butter "manufactured in Arizona" were "supplied" by the Associated Dairy Products Company, Glendale, Ariz., at frequent intervals from December 1943 through May 1945.[4] The butter was stored at a freezing temperature until analyzed. The results for 25 samples of butter, tested throughout a period of 16 months, are shown in table 19. There is apparently no seasonal variation in the vitamin A potency of the butter produced in Arizona.

[4] FARRANKOP, H. THE VITAMIN A CONTENT OF ARIZONA BUTTER. Arizona Agr. Expt. Sta. Mimeographed Report 74, 2 pp. 1945. (Processed.)

TABLE 19.—*Vitamin A potency of creamery butter produced in Arizona*

Date of delivery of sample	Potency per pound: Not corrected for loss in analysis	Potency per pound: Corrected for loss of vitamin A in analysis
1943	*I. U.*	*I. U.*
Dec. 22	15,786	
1944		
Jan. 4	17,302	
Jan. 24	19,299	
Feb. 4	18,297	
Feb. 18	19,880	
May 12	18,692	
June 1	18,251	
June 9	17,662	
June 26	18,142	
July 3	17,250	
July 10	18,310	
Aug. 1	17,604	
Aug. 15	16,897	
Aug. 28	17,854	
Sept. 11	16,600	
Sept. 27	16,098	
Oct. 10	16,420	
Nov. 14	16,805	
Dec. 7	14,754	
1945		
Jan. 2	16,336	
Jan. 19	16,708	
Feb. 9	18,892	
Mar. 2	18,221	
Apr. 27	16,879	
May 14	17,457	
Average, December–February, both years	17,473	
Average, March–November, both years	17,446	
Average, annual [1]	17,457	[2] 18,410

[1] All averages are unweighted; the December–February production was 16.9 percent, and the March–November production 83.1 percent of the annual output.
[2] Calculated by using average correction of other butters of similar potency.

SUMMARY OF ROCKY MOUNTAIN AND PACIFIC COAST STATES.—A summary of the results on the vitamin A potency of the butter produced in the Rocky Mountain and Pacific Coast States that participated in this study is given in table 20.

SUMMARY OF RESULTS ON THE VITAMIN A POTENCY OF CREAMERY BUTTER PRODUCED IN THE UNITED STATES

The results on the vitamin A potency of the creamery butter produced in the United States are summed up in table 21. The States participating in this survey produce 64 percent of the creamery butter produced in the United States; and their product undoubtedly may be considered as representative of practically all the creamery butter produced in this country. Of this butter, about 35.4 percent may be considered "winter" butter with an average vitamin A potency of about 10,500 or 11,200 I. U. per pound. About 64.6 percent of the creamery butter produced in this country is "summer" butter, produced by cows on pasture. This has an average vitamin A potency of 17,000 or 18,000 I. U. per pound; and it is likely that 15,000 I. U. per pound is as close as one can approximate the average vitamin A potency of the total output of creamery butter now being produced in the United States.

TABLE 20.—*Summary of the results on the vitamin A potency of butter produced in 7 Rocky Mountain and Pacific Coast States*

State	Annual production (average for 1942–43) [1]	Winter production	Summer production	Mean potency per pound of butter: Winter butter, Not corrected for loss in analysis	Winter butter, Corrected for loss of vitamin A in analysis	Summer butter, Not corrected for loss in analysis	Summer butter, Corrected for loss of vitamin A in analysis	Annual, Not corrected for loss in analysis	Annual, Corrected for loss of vitamin A in analysis
	Million pounds	*Percent*	*Percent*	*I. U.*	*I. U.*	*I. U.*	*I. U.*	*I. U.*	*I. U.*
Washington	32.0	16.3	83.7	11,355	12,000	18,640	19,580	17,458	18,344
Oregon	28.2	30.9	69.1	12,340	13,000	17,700	18,560	15,740	16,528
California	43.2	20.6	79.4	14,414	15,200	18,200	19,140	17,420	18,330
Idaho	37.1	37.1	62.9	12,621	13,312	17,338	18,197	15,587	16,384
Montana	12.7	29.7	70.3	17,550	18,636	20,752	22,018	19,801	21,014
Wyoming	3.1	34.8	65.2	9,170	9,750	12,870	13,600	11,586	12,263
Arizona	1.3	16.9	83.1	17,473	18,420	17,446	18,390	17,456	18,410
Total or mean [2]	157.6	26.4	73.6	13,170	13,900	18,150	19,080	16,838	17,710

[1] Total annual butter production for the 11 States in this region (Washington, Oregon, California, Idaho, Montana, Colorado, Wyoming, Nevada, New Mexico, Utah and Arizona) was 194,600,000 pounds, 1942–43.
[2] Weighted for production in participating States.

TABLE 21.—*Summary of results on the vitamin A potency of creamery butter produced in the United States*

ANNUAL PRODUCTION OF BUTTER (AVERAGE, 1942–43)

Area	All States in area		Participating States in area		Winter butter	Summer butter
	Million pounds	*Percent* [1]	*Million pounds*	*Percent* [1]	*Percent* [2]	*Percent* [2]
North Central States	1,324.9	77.1	934.1	54.4	37.9	62.1
South Atlantic and South Central States	151.1	8.8	6.6	.4	25.5	74.5
Rocky Mountain and Pacific States	194.6	11.3	157.6	9.2	26.4	73.6
Total or mean	1,670.6	97.2	1,098.3	64.0	35.4	64.6

MEAN POTENCY PER POUND OF BUTTER

Area	Winter butter: Not corrected for loss in analysis	Winter butter: Corrected for loss of vitamin A in analysis	Summer butter: Not corrected for loss in analysis	Summer butter: Corrected for loss of vitamin A in analysis	Annual: Not corrected for loss in analysis	Annual: Corrected for loss of vitamin A in analysis
	I. U.	*I. U.*	*I. U.*	*I. U.*	*I. U.*	*I. U.*
North Central States	10,240	10,880	17,150	18,120	14,540	15,390
South Atlantic and South Central States	10,640	11,270	16,480	17,360	14,990	15,807
Rocky Mountain and Pacific States	13,170	13,900	18,150	19,080	16,838	17,710
Total or mean	10,530	11,170	17,210	18,160	14,850	15,680

[1] Percentage of average National output for 1942 and 1943—i. e. of 1,718,600,000 pounds.
[2] Weighted by using production in all States in each group and seasonal distribution for participating States.

THE VITAMIN A POTENCY OF RENOVATED BUTTER

Maryland.—In his 1943 report of work done in connection with this survey, C. W. England, then of the Maryland Agricultural Experiment Station, reported results on the vitamin A potency of renovated butter produced in Maryland. Samples of butter collected in May and July of that year had a potency of 13,750 I. U. per pound.

THE EFFECT OF STORAGE ON THE VITAMIN A POTENCY OF BUTTER

The laboratories at the Wisconsin, North Carolina, Ohio, Minnesota, California, Montana, and Mississippi Agricultural Experiment Stations investigated the effect of storage on the vitamin A potency of butter. Samples of butter were handled and stored under various practical conditions. The results indicate that both carotene and vitamin A are very stable in butters under these conditions; that little if any loss of vitamin A potency occurs during the periods that commercial butters are ordinarily stored; and that, insofar as the effect of storage is concerned, one would not expect the average vitamin A potency of the butter sold on the retail markets in this country to be significantly different from that of the butters produced in the creameries of the country. The results from each State on the effect of storage are briefly as follows:

Wisconsin.—Berl and Peterson (*4*) stored four 64-pound tubs of butter at −21° to −23° C. The tubs were unparaffined, lined with parchment paper, and closed with wooden lids. There was no change in either the vitamin A or carotene after storage for 8 months.

They also stored seventy 1-pound packages of butter in original paper wrappings and cartons for 5 months. Ten of these samples were then analyzed. These samples showed no loss of either vitamin A or carotene.

North Carolina.—The North Carolina Agricultural Experiment Station did not report in detail the results of its studies on the storage of butter, but said:

> Butters collected during August and November 1943 showed no loss of carotene or vitamin A when stored at 10° F. for 3 months or 6 months. As a matter of fact, the carotene values in all cases showed an embarrassing increase of 0.1 to 0.3 microgram per gram.

Ohio.—At the Ohio Agricultural Experiment Station 156 samples of butter were stored at 0° F. for 6 months, and 96 of these samples were stored for 1 year. The average vitamin A content of the 156 samples when fresh was 4.97 micrograms, and after storage for 6 months it was 5.02 micrograms per gram; the carotene content was 5.30 micrograms per gram and 5.23 micrograms per gram, respectively. With the 96 samples stored for 12 months, the vitamin A in the beginning was 4.53 micrograms per gram and after storage it was 4.62 micrograms per gram; the carotene content was 4.51 micrograms per gram and 3.84 micrograms per gram, respectively. The station report says:

> No loss in vitamin A occurs after storage at 0° F. for 6 months to 1 year but carotene is slightly lowered. There is, therefore, little change in vitamin A potency expressed in International Units per pound.

MINNESOTA.—The Minnesota Agricultural Experiment Station reported (*17*) as follows:

One 10-pound tub of butter was received from each region each month during the first 6 months of 1943. After withdrawal of a sample for immediate analysis, the tubs were placed in storage at −10° F., and other samples were withdrawn and analyzed after storage periods of 3 and 6 months. The results of this study show that the carotene content of these butters was little affected by storage. Statistical analysis revealed that the differences attributable to the effects of storage were insignificant. In the case of the vitamin A content, the very disturbing anomalous increases noted in the February, May, and June samples after storage must be due to an uncontrolled error in the experiment. For the data as a whole, however, the differences in vitamin A content due to storage are statistically insignificant. Certainly there is no evidence of deterioration in potency during storage.

The mean vitamin A and carotene contents of the 52 tubs of butter before and after storage are shown in table 22.

TABLE 22.—*Effect of storage on the vitamin A potency of Minnesota butter* [1]

Storage period	Vitamin A per gram	Carotene per gram
	µg.	*µg.*
Fresh	5.16	2.97
3 months	5.38	2.97
6 months	5.50	2.93

[1] Weighted mean of 52 tubs.

CALIFORNIA.—The California Agricultural Experiment Station purchased six brands of butter at three different markets at Davis, Calif., on June 2, 1943, and determined the carotene and vitamin A content at the time of purchase and also after 7 months' storage. The effect of storage on the vitamin A potency of the butter is shown in table 23.

TABLE 23.—*Effect of storage on vitamin A potency of butter purchased on Davis, Calif., markets, June 2, 1943*

Market and brand of butter	Butter when purchased			Butter after 7 months' storage		
	Vitamin A per gram	Carotene per gram	Vitamin A potency per pound [1]	Vitamin A per gram	Carotene per gram	Vitamin A potency per pound [1]
	µg.	*µg.*	*I. U.*	*µg.*	*µg.*	*I. U.*
Market No. 1:						
Brand A	8.24	7.78	20,850	6.35	7.27	17,030
Brand B	7.09	9.09	19,750	6.64	8.84	18,830
Brand C	7.46	7.34	19,100	6.47	7.02	17,070
Market No. 2:						
Brand D	6.01	1.43	12,000	5.56	1.48	11,230
Brand A	7.10	6.38	17,760	6.14	6.69	16,225
Market No. 3:						
Brand E	8.88	4.08	19,220	8.95	4.15	19,390
Average	7.46	6.02	18,113	6.70	5.91	16,629

[1] 0.25 micorgram per gram of vitamin A = 1 International Unit; 0.6 microgram per gram of carotene = 1 International Unit.

In reporting these results, the California Agricultural Experiment Station said:

> Discrepancies in results preclude positive statements concerning storage losses. One-pound prints, from which samples must be taken for analyses and scoring, even when care is exercised in rewrapping, can scarcely be expected to yield the same results in storage as tubs of butter similarly tested. Significant losses in 6 months and 1 year of storage occurred in the butter of only one creamery. This creamery has only a small operation and frequently the cream is 4 days old at the time of churning and requires fairly heavy neutralization. Incidentally, when calcium and magnesium lime is used as a neutralizer, clear extractions are difficult to obtain unless a polyphosphate (such as Calgon) is added during saponification.

Later the California station reported the results in table 24, as "representative of a large number of samples which have been followed through storage." The average loss of vitamin A during storage was 7.4 percent for the spring samples, 7.6 percent for the summer samples, and 5.5 percent for the fall samples. The average loss for all samples was 7 percent. These averages would seem to indicate a small—but significant—loss. With the carotene there was an average gain of 3.6 percent in the spring samples, and losses of 5 percent and 11 per cent in the averages of the summer and fall samples. The mean of the differences in the carotene content of all the samples before and after storage probably does not indicate a significant change in this constituent. A sample of "poor" winter butter containing 6.61 micrograms of vitamin A and 7.54 micrograms of carotene per gram before storage had 4.62 and 2.74 micrograms, respectively, after 8 months of storage and 1.17 and 0.7 micrograms after 1 year of storage.

TABLE 24.—*Effect of storage on the vitamin A potency of California butter*

Region	Spring butter				Summer butter				Fall butter			
	Initial		After 8 months' storage at 0° F.		Initial		After 8 months' storage at 0° F.		Initial		After 9 months' storage at 0° F.	
	Vitamin A per gram	Carotene per gram	Vitamin A per gram	Carotene per gram	Vitamin A per gram	Carotene per gram	Vitamin A per gram	Carotene per gram	Vitamin A per gram	Carotene per gram	Vitamin A per gram	Carotene per gram
	µg.	*µg.*	*µg.*	*µg.*	*µg.*	*µg.*	*µg.*	*µg.*	*µg.*	*µg.*	*µg.*	*µg.*
A	7.95	12.11	7.64	12.68	6.90	8.21	6.61	8.25	6.84	11.34	6.73	9.56
B	6.83	10.20	5.99	11.23	5.62	4.84	6.29	4.23	5.59	4.01	5.05	3.32
C	9.51	9.08	8.91	9.03	7.88	5.55	5.77	5.88	7.85	4.31	6.88	4.41
D	7.48	10.23	6.52	10.20	6.58	6.96	6.70	7.00	5.87	7.16	5.45	6.03
E	------	------	------	------	7.76	5.75	7.75	4.39	7.41	5.10	7.27	5.15
F	5.34	2.38	5.34	2.44	7.75	3.98	6.14	3.78	5.77	4.35	5.82	3.55
Average	7.42	8.80	6.88	9.12	7.08	5.88	6.54	5.59	6.56	6.04	6.20	5.34

MONTANA.—The average carotene and vitamin A contents of 15 samples of butter analyzed by the Montana Agricultural Experiment Station before and after storage are shown in table 25. It would be difficult to be certain that there was a significant change in either carotene or vitamin A as a result of the storage of these samples; certainly there was no obvious change as a result of storage for 90 days; and one might even question the significance of the differences between

the carotene and vitamin A contents of the fresh samples as compared with those of the samples stored for 180 days.

The Montana Agricultural Experiment Station said of these results:

> There were no appreciable changes in the beta-carotene or the vitamin A content of the butters stored for 90 days at 0° F. Butters stored at 0° for 180 days showed no significant changes in the beta-carotene values, but some decreases in the vitamin A values appeared in most of the samples, particularly for those butters produced in the "fall."

TABLE 25.—*Average vitamin A and carotene content of 15 samples of butter analyzed before and after storage (Montana)*

Storage time and temperature	Carotene per gram of butter	Vitamin A per gram of butter
	µg.	*µg.*
Fresh	5. 62	10. 10
30 days at 40° F	5. 46	12. 69
90 days at 0° F	5. 67	10. 73
180 days at 0° F	5. 28	9. 01

MISSISSIPPI.—At the Mississippi Agricultural Experiment Station the monthly samples of butter in three creameries were analyzed immediately after the butter was made and again after storage for 15 and 30 days at 45° F. The results for butters made from variously treated creams are summarized in table 26. There apparently was practically no change in either the carotene or vitamin A. In addition to the above samples, other samples were taken during certain months and stored for 5 months at 0° F. before being analyzed. They were then analyzed immediately and again after storage for 15 and 30 days more at 45°. The results for these butters, also made from variously treated creams, were substantially similar to those in table 26.

TABLE 26.—*Change in carotene and vitamin A content of butter after storage for 15 and 30 days at 45° F. (Mississippi)*

Treatment of the cream used in making the butter	Analyzed immediately		After 15 days' storage at 45° F.		After 30 days' storage at 45° F.	
	Carotene per gram	Vitamin A per gram	Carotene per gram	Vitamin A per gram	Carotene per gram	Vitamin A per gram
	µg.	*µg.*	*µg.*	*µg.*	*µg.*	*µg.*
Butter made from sweet cream, separated on farm, delivered to plant, pasteurized at 155° F. for 30 minutes	[1] 6.2	[2] 6.06	[1] 6.01	[2] 6.02	[1] 5.9	[2] 6.04
Butter made from cream separated on farm, delivered to plant sour, neutralized, pasteurized at 185°–200° F.	[1] 5.4	[2] 5.62	[1] 5.1	[2] 5.78	[1] 5.4	[2] 5.57
Butter made from sweet cream, separated from milk at plant, pasteurized at 155° F. for 30 minutes	[2] 6.2	[3] 5.96	[2] 5.8	[3] 5.91	[2] 5.8	[3] 5.87
Average	5.9	5.88	5.6	5.90	5.7	5.83

[1] Samples taken during 15 months are included in these averages.
[2] Samples taken during 14 months are included in these averages.
[3] Samples taken during 13 months are included in these averages.

VITAMIN A POTENCY OF THE BUTTER SOLD ON RETAIL MARKETS

The Texas Agricultural Experiment Station conducted independently an investigation of the vitamin A potency of butter sold on the retail markets in that State. The Alabama Agricultural Experiment Station studied the vitamin A potency of creamery and "country" butter. Most samples of the creamery butter were obtained on the local markets; some of them were produced in creameries in Alabama. The "country" butter was produced locally. The New York (Cornell) and Pennsylvania experiment stations conducted very systematic investigations of butter (nationally known brands and locally produced butter) sold on retail markets.

Since butters that are produced under summer or winter feeding conditions may be sold for several months thereafter on retail markets, and the extent to which this occurs may vary from year to year, it is difficult to select random samplings of market butters that are comparable with those produced in the creameries of the country. From the results in the preceding section on the effect—or lack of effect—of commercial methods of storage and handling on the vitamin A potency of butter, it is obvious that with the same samples of butter, followed through from the creamery to the local market and subjected to ordinary conditions by the consumer, one would expect little if any loss in vitamin A potency. The following results on the vitamin A potency of random samples of butter, purchased on retail markets, agree with the conclusion that there is in general no significant or demonstrable difference in vitamin A potency between these butters and those produced in the creameries of the country. The results on the vitamin A potency of market butters follow.

TEXAS.—Prior to the time this Nation-wide cooperative survey was started, Kemmerer and Fraps of the Texas Agricultural Experiment Station were engaged in a study of the vitamin A potency of commercial butters sold in Texas. Their results have since been published (*18*). These workers used a different method of determining the vitamin A from that used in this survey. (See Physiological Significance of Analytical Results, p. 34.) They report results on 62 samples of market butters sold in Texas during the year 1942 and in January 1943. The average monthly potency per pound of these samples (obtained by multiplying the reported International Units per gram by 454) is shown below:

Samples collected:	*International Units of vitamin A per pound of butter*
1942:	
January	16, 796
March	15, 888
April	19, 520
July	17, 250
August	13, 618
September	17, 703
October	14, 027
November	14, 980
1943:	
January	17, 250
Average of monthly averages	16, 344

The average of these average monthly potencies is 16,344 International Units per pound. If we allow for the differences due to the analytical methods used (see table 35) these results might be considered in good agreement with the creamery butters studied in such States as Wisconsin, Minnesota, Iowa, and others.

New York (Cornell).—This station studied the vitamin A potency of the butters sold on the retail markets in Syracuse (population 206,000) which is served by 650 grocery stores, including the chain stores (*14*). Areas were selected in the city to represent different levels of consumer income as well as differences in the size and type of stores. In each store visited, a sample of each brand of butter for sale was purchased. Thus a number of samples of the same brand were obtained from different stores. The complete results of this very interesting survey will be published later by the Cornell workers. The results that bear on the over-all average vitamin A potency of market butters are shown below. These results, which are not corrected for loss in analysis, compare very well with the same figures for the creamery butters produced in such States as Minnesota, Wisconsin, Iowa, and others.

Samples collected:	*International Units of vitamin A per pound of butter*
1943:	
April	15, 781
June 1–2	16, 155
June 28–29	19, 916
August	20, 870
September	17, 770
October	19, 372
November	16, 652
Average of 1943 monthly averages (unweighted)	18, 053
1944:	
January	12, 998
February	10, 700
March	12, 220
April	9, 294
May	10, 553
Average of 1944 monthly averages (unweighted)	11, 147
Average, all monthly averages (unweighted)	15, 180

Pennsylvania.—In table 27 are shown the individual variations in the vitamin A potency that may occur with random samples of butter obtained on retail markets at different times of the year. These samples were collected and analyzed by the Pennsylvania State College. The samples (about 100) represented 6 brands of butter, of which 5 were nationally advertised brands and 1 was the product of a local creamery.

The difficulties encountered in comparing the potency of the butter sold on retail markets in this country with the potency of the butter as produced in the creameries are very apparent in these data from Pennsylvania. Butters produced under summer conditions were obviously sold on the market in January and even in February; some of the product produced under winter conditions was sold on these markets even in June; and these conditions undoubtedly vary from year

to year, depending on the extent of the carry-over of the summer and winter product. Also, it must be realized that the results in table 27, except those in the last column, are not averages of large numbers of samples as in the case of much of the data in this report, but are the "high" and "low" values obtained. These data are interesting in showing the variation in the potency of butters that may be purchased by the consumer.

The vitamin A potency of the butters purchased during January to May, inclusive, averages 10,570 I. U. per pound; that for those purchased during June to December averages 18,185 I. U. per pound. The unweighted yearly average is 15,100 I. U. per pound. These data, which are not corrected for loss in analysis, agree exceedingly well with those obtained by the Cornell workers for the butters purchased on the retail markets in Syracuse and, like those results, they compare very well with the vitamin A potency of the creamery butters produced throughout this country.

Table 27.—*The monthly range of vitamin A potency of 6 commercial butters as sold on the retail market in Pennsylvania between September 1943 and August 1944 (not corrected for loss in analysis)*

Monthly range	Vitamin A potency per pound of sample—						Average monthly potency
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	
	I. U.	*I. U.*	*I. U.*	*I. U.*	*I. U.*	*I. U.*	*I. U.*
October:							
High	21, 556	20, 357	21, 260	21, 738	22. 585	23, 520	20, 442
Low	19, 807	18, 644	18, 963	13, 095	20, 462	23, 315	
November:							
High	22, 304	21, 918	19, 898	22, 205	23, 242	26, 013	21. 008
Low	20, 156	19, 852	18, 696	18, 342	18, 445	21, 020	
December:							
High	22, 095	18, 310	19, 105	18, 010	18, 014	23, 424	17, 598
Low	14, 904	16, 057	16, 888	13, 442	11, 416	19, 514	
January:							
High	20, 883	13, 407	12, 589	15, 754	11, 972	13, 698	13, 134
Low	13, 680	11, 669	9, 639	13, 378	11, 532	9, 463	
February:							
High	16, 693	10, 349	9, 881	11, 154	10, 930	12, 698	11, 261
Low	10, 610		9, 275	11, 075	10, 396	10, 805	
March:							
High	10, 093	10, 598	8, 386	10, 215	10, 534	10, 388	9, 540
Low	9, 021	10, 397	8, 005	9, 647	9, 703	7, 494	
April:							
High	8, 459	11, 500	8, 640	10, 427	9, 657	10, 655	9, 355
Low	8, 127	8, 582	7, 914		9, 140	9, 807	
May:							
High	9, 762	10, 728	15, 254	10, 683	10, 932	9, 398	10, 051
Low	8, 004	8, 431	9, 398	9, 807	9, 475	8, 731	
June:							
High	8, 731		18, 192	22, 110	21, 566	8, 354	13, 727
Low	6, 888		17, 192	9, 716	10, 792		
July:							
High	15, 753	16, 436	18, 101	21, 879	19, 028		18, 290
Low			15. 403	21, 428			
August:							
High	19, 554	18, 455	18, 688	22, 848	20, 504	18, 886	19, 357
Low	19, 178	17, 182	17, 288	20, 985			
September:							
High	21. 072	19, 103	18. 978	21, 945	20, 904	20, 950	19, 914
Low	20, 559	16. 536	18, 071	20, 349	19, 612	20, 885	
Average	15, 126	14, 926	14, 821	16, 097	15. 039	15, 451	
Average, unweighted, January–May							10, 669
Average, unweighted, June–December							18, 618
Average, unweighted, for year							15, 307

ALABAMA.—In tables 28 and 29 are shown the results on the vitamin A potency of creamery and country butter purchased on retail markets in Alabama. Some of the creamery butter (table 28) and all of the country butter (table 29) was produced in Alabama.

TABLE 28.—*Vitamin A potency of creamery butter purchased on retail markets in Alabama* [1]

Date of purchase	Carotene per gram of butter	Vitamin A per gram of butter	Potency per pound: Not corrected for loss in analysis	Potency per pound: Corrected for loss of vitamin A in analysis
1943	*µg.*	*µg.*	*I. U.*	*I. U.*
January	5.31	4.82	12,770	13,416
February	2.61	4.24	9,674	10,246
March	2.48	4.66	10,336	10,962
April	3.33	4.71	11,071	11,705
May	2.41	5.61	12,011	12,767
June	4.31	6.01	14,154	14,980
July	3.72	3.75	9,624	10,127
August	4.88	5.27	13,260	13,970
September	4.59	4.95	12,461	13,126
October	5.01	5.71	14,159	14,926
November	4.55	6.29	14,861	15,705
December	3.83	5.03	12,030	12,703
Average, December to March [2]			11,202	11,832
Average, April to November [2]			12,700	13,413
Average, annual [2]			12,201	12,886
1944				
January	3.37	6.11	13,647	14,483
February	2.50	5.64	12,134	12,907
March	2.20	4.89	10,545	11,214
April	3.46	5.51	12,624	13,379
May	3.76	5.74	13,269	14,056
June	3.47	5.29	12,230	12,952
July	6.17	6.97	17,323	18,278
August	5.35	6.40	15,670	16,548
September	5.58	5.99	15,098	15,917
Average, December to March			12,109	12,868
Average, April to September			14,369	15,188
Average of all months			13,616	14,415

[1] Some of the samples were produced in Alabama creameries, and the potency of this butter in 1943 was probably affected by drought conditions during the summer of that year.
[2] Unweighted.

POSSIBILITIES FOR IMPROVING THE VITAMIN A POTENCY OF MILK AND BUTTER

There is ample experimental evidence that the vitamin A potency of milk and butter is, within certain limits, readily changed by increasing or decreasing the quantity of carotene in the cow's ration. The large increases in the vitamin A potency of summer butter over winter butter produced in 14 of the 16 States for which the results are given in tables 1 to 21 are no doubt largely, if not exclusively, associated with the increase in the carotene intake when the cows were put on pasture and vice versa. The Minnesota results (table 1) show how rapidly this change may occur in the butter when the cows are put on pasture in the spring. The Louisiana results for July 1944 (table 10) also show how completely this change may be reversed by the effect of drought on the pasture in summer. The results from Oregon suggest that, by suitable feeding, "summer" butter may be produced in winter.

Table 29—Vitamin A potency of country butter obtained on the retail market in Alabama

Month of purchase	1943		1944		Potency per pound (average 1943–44)[1]	
	Carotene per gram of butter	Vitamin A per gram of butter	Carotene per gram of butter	Vitamin A per gram of butter	Not corrected for loss of vitamin A in analysis	Corrected for loss of vitamin A in analysis
	µg.	*µg.*	*µg.*	*µg.*	*I. U.*	*I. U.*
January	3.64	4.49	1.17	4.48	9,952	10,571
February	4.05	4.67	1.08	5.06	10,774	11,434
March	4.41	4.81	2.24	6.53	12,810	13,582
April	3.86	5.43	3.16	7.55	14,440	15,320
May	5.12	6.09	9.00	6.10	16,408	17,235
June	5.56	5.09	7.08	5.24	14,160	14,861
July	5.54	4.90	5.56	9.16	16,964	17,920
August	5.31	5.55	6.38	6.08	14,990	15,769
September	5.43	4.94			13,078	13,738
October	5.54	4.89			13,070	13,725
November	5.63	5.39			14,045	14,767
December	1.76	4.72			9,901	10,535
Mean, December–March [2]					10,728	11,393
Mean, April–November [2]					14,740	15,511
Mean, annual [2]					13,544	14,284

[1] The potencies for the 2 years were averaged without weighting.
[2] Weighted for 1943 monthly production.

In table 30 are shown results, similar to those from Minnesota, that were obtained by the Bureau of Dairy Industry when cows at the Beltsville, Md., station were put on pasture and kept on pasture throughout a season in which the rainfall varied considerably. It will be noted that the vitamin A potency of the butter from the Holstein cows increased more than 100 percent during the first 2 weeks (April 21 to May 8) the cows were on pasture, and that it subsequently varied from 14,270 to over 22,600 I. U. per pound while these cows were on pasture, depending on the rainfall and consequent conditions of the pasture.

The fluctuation in vitamin A potency was not so great in the butter from the Jerseys as in that from the Holsteins. It increased about 50 percent during the first 2 weeks the cows were on pasture (April 21 to May 8); and varied from 13,900 to nearly 18,000 I. U. per pound with the various pasture conditions during the season. Similar results are shown for cows for the summer and fall of 1943.[5] The results in table 30 show that practically the whole range of variation that occurred in the vitamin A potency of the butter from State to State (tables 1 to 21) may occur with the same herd under various feeding conditions; and that the same variation noted from State to State in the vitamin A potency of summer butter may occur in the same herd either from season to season, or between butters produced at different times during the same season, as a result of differences in pasture conditions due to differences in rainfall.

[5] Unfortunately, samples were not obtained during the severe drought in midsummer of 1943.

TABLE 30.—*Vitamin A potency of butter and milk produced by cows at Beltsville, Md.*

Description of cow groups and the feeding conditions that affected their source of vitamin A	Vitamin A potency of butter per pound [1]		Vitamin A potency of milk per quart [1]	
	Holstein	Jersey	Holstein	Jersey
1943	*I. U.*	*I. U.*	*I. U.*	*I. U.*
June.—Cows on good pasture	19,580	18,280		
Fall.—Cows just before going off pasture that had not recovered entirely from the effect of earlier drought	15,680	13,620		
1944				
Late spring.—Cows on barn-fed rations of grain, corn silage, and alfalfa hay (possibly top U. S. No. 2)	12,680	10,160		
April 21.—Groups (8 Holsteins and 8 Jerseys) of barn-fed cows just before going out on pasture; hay not quite so good as above	9,000	10,210	888	1,239
May 8.—Same groups as above, on pasture 2 weeks. Rainfall in April had been normal; there was abundant, immature grass	18,560	15,720	1,698	1,970
May 26–28.—Same groups as above, on pasture; May rainfall 1.2" (normal 3.5"); pasture still abundant and mostly immature	20,000	17,477	1,815	2,190
June 9.—Same groups as above; June rainfall 1.9" (normal 4"); grass becoming dry, some overripe; cattle "eating-over" where they had grazed before	15,900	15,590	1,613	2,006
June 23.—Same groups as above	16,260	14,700	1,648	1,887
July 11.—6 of above Holsteins and 7 of the Jerseys	14,270	13,900	1,372	1,854
July 13.—0.5" rain fell; July 19, 1" rain fell; July rainfall 1.8" (normal 3.5")				
July 25.—5 of above Holsteins and 6 of above Jerseys	16,051	14,422	1,581	1,904
August 8.—5 of above Holsteins and 5 Jerseys; pasture; 6" rain fell on August 1 and 2, and 6.58" total for month (normal about 4.53")	18,364	16,800	1,980	2,252
August 22.—Pasture good; 4 of above Holsteins and 5 Jerseys	19,383	16,044	2,019	2,155
Sept. 6.—Pasture good, plenty of green grass (group same as August 22)	21,650	17,973	2,534	3,053
Sept. 19.—4 of above Holsteins and 4 Jerseys; pasture good, plenty of green grass. Rainfall in September 6.53" (normal 3.46")	22,650	17,190	2,582	3,072

[1] Not corrected for loss of vitamin A in analysis.

The results for the vitamin A potency of winter butter shown in figure 1 were also obtained at Beltsville.[6] They show that the feeding of an aliquot of the same crop of alfalfa as silage instead of as hay may double the vitamin A potency of winter butter—the alfalfa silage apparently supplying from 4 to 20 times as much carotene per unit of dry matter as the hay. The results in figure 1, which are similar to those obtained in the Nation-wide survey, show also that feeding alfalfa hay or corn silage that is low in carotene content produces butter of low vitamin A value.

One unusual cow on the alfalfa-silage ration produced butter in January, February, and March with an average vitamin A potency of 20,400 I. U. per pound, and while she was on pasture she produced butter with about 30,000 I. U. per pound. These results are not included in the data given in figure 1, but they suggest that the individuality of the cow—possibly breeding as well as feeding—may affect the vitamin A potency of the butter produced.

[6] This work was conducted by the Division of Nutrition and Physiology of the Bureau of Dairy Industry, in cooperation with the Division of Forage Crops and Diseases and the Division of Agricultural Engineering of the Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

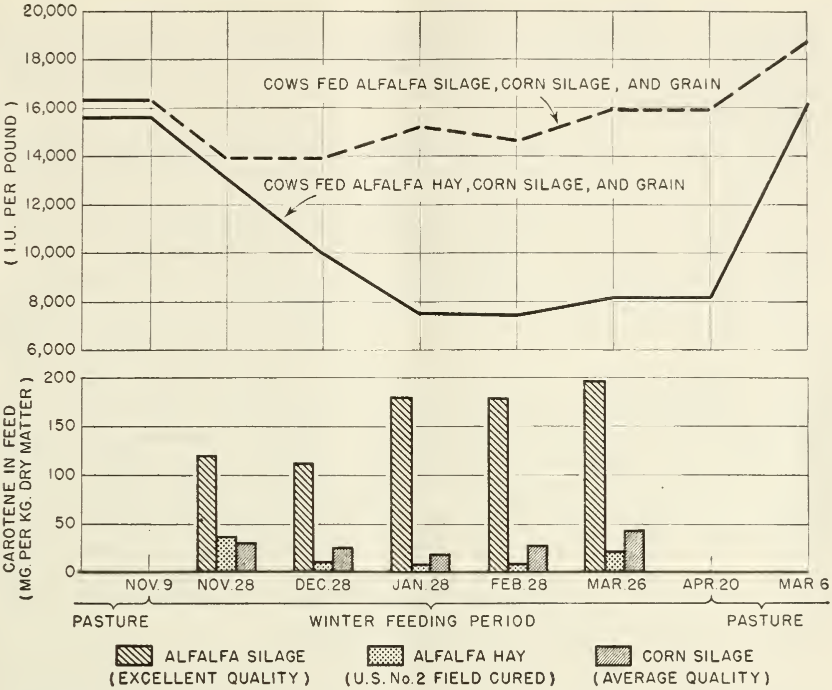


FIGURE 1.—Effect of winter feed on the vitamin A potency of butter.

The question as to whether or not there are conditions other than the quantity of carotene in the feed that affect the efficiency of the utilization of carotene by the cow has not been investigated adequately. It is obvious, however, from the above work, and from a large amount of other work in other laboratories, that any condition which tends to increase the lushness of pasture or to increase the carotene content of winter-fed forages—either silages or hays—can very greatly increase the vitamin A potency of milk and butter. Hays that are cured in such a way as to retain their green color are rich in carotene, whereas hays that have lost their green color in curing may be very poor sources of this precursor of vitamin A. Alfalfa hays may be rich sources of vitamin A or practically worthless in this respect; timothy hays may vary several-fold in their carotene content; and, although there may be no loss of carotene in preserving corn silage in a large concrete silo for months, the carotene content of corn silage may vary several-fold depending on the carotene content of the corn plant from which it is made. The carotene content of the corn plant decreases rapidly with an increase in maturity at certain stages and with a decrease in the greenness of the plant when it is cut.

Unquestionably, much improvement in the vitamin A potency of milk and butter could be made immediately and economically if good feeding practices were used more widely. The improvement in the potency of the butter from the cows fed alfalfa silage in place of alfalfa hay (fig. 1) was brought about without an increase in labor cost per

unit of feed. Much improvement may be brought about by the development of practical and economical methods of producing and processing forages and by improvements in pasture management. Investigations along these lines are now in progress in many States. Similar studies are being conducted cooperatively by the Bureau of Plant Industry, Soils, and Agricultural Engineering and the Bureau of Dairy Industry of the United States Department of Agriculture. It has been recommended that the State stations and Federal laboratories cooperate in a program to improve the vitamin A potency of butter just as they have cooperated in the present survey.

The beta-carotene group in plant carotenoids is the only source of vitamin A. A very large amount of this material occurs in the roughages available as feed for the cow. The farmer may waste this raw material or he may conserve it for human use by suitable farm practices. Increasing the carotene content of the diet of the normal cow does not increase her milk yield; and a normal cow would need much more carotene to produce milk as rich in vitamin A as average summer milk than she would need merely to maintain normal health or to bear a normal calf. Therefore, any improvement in farm practice which is recommended as a means of increasing, beyond certain limits, the vitamin A potency of milk for commercial purposes, must at the same time have other advantages as a method of producing feed, or else be compensated for by "trade" considerations. The former possibility needs further investigation.

In reporting the results obtained in their study of the vitamin A in butter, Palmer and Jenness of the Minnesota Agricultural Experiment Station presented an interesting estimate of the total quantity of vitamin A and carotene in the butter produced in Minnesota in 1943. It was 1,800 pounds of vitamin A and 1,100 pounds of carotene. These amounts together would supply the total annual vitamin A requirements of 2,500,000 people.

It has been estimated that the total amount of carotene and vitamin A in the milk and milk products consumed in the United States is adequate to supply the total vitamin A requirements of 42,000,000 people. This is a large contribution by the dairy industry to the national need for vitamin A. If, by proper feeding, the vitamin A potency of winter milk were increased to that of average summer milk it would increase the total output of vitamin A in milk and milk products by about 27 percent, or sufficiently to supply the total vitamin A requirements of 11,000,000 more people. To what extent this may be practical depends on the results that may be obtained with methods of conserving the carotene in hays and silages. Some improvement in summer butter is also possible. The conditions which produce a lush pasture rich in carotene do at the same time produce more feed and more milk, as well as better milk.

APPLICATION OF THE RESULTS TO MARKET MILKS

Calculations based on the results in table 21 indicate that the average vitamin A potency of the fat in winter milk is 28.7 or 30.4 (about 29.5) I. U. per gram and in summer milk 46.9 or 49.5 (about 48.2) I. U. per gram. The proportionate amounts of winter and summer milk consumed by the people in this country are probably

close to 40 percent and 60 percent, respectively, of the annual consumption of milk.[7] On this basis one may calculate the average vitamin A potency of milk (4-percent fat content) to be approximately as follows:

	International units of vitamin A per quart
Winter milk (milk produced by cows on barn rations)	1, 120
Summer milk (milk produced by cows on pasture)	1, 824
Average of milk consumed annually	1, 540

The "disappearance" of milk "for domestic human consumption in all forms" in the United States in 1941 was equivalent to 1.052 quarts per capita per day (*26*, p. 471). This amount of milk apparently furnished slightly over 1,600 I. U. per capita per day for the total population.

In table 30 is shown the vitamin A potency of milk produced at the end of the winter season and under various summer-feeding conditions at Beltsville. At the end of the winter season the Holstein milk reached a low vitamin A potency of about 900 I. U. per quart and the Jersey milk a low of 1,240 I. U. per quart. During the summer, under various pasture conditions, the Holstein milk varied from 1,370 to 2,580 I. U. per quart, whereas the Jersey milk varied from about 1,850 to 3,070 I. U. per quart.

Berl and Peterson, in publishing their results of this survey (*4*), compared them with the earlier results of Dornbush, Peterson, and Olson (*11*) for milks. Dornbush, Peterson, and Olson (*11*) give the average potency of "market" milk as 1,088 U. S. P. units per quart in the winter, and 1,906 U. S. P. units per quart in the summer. Their "market" milks contained an average of 3.5 percent of fat. The potencies of their market milks are, therefore, about 18 percent higher than would be calculated from the average results obtained in this survey. This may be accounted for in part by the fact that Dornbush, Peterson, and Olson used a different method in obtaining their milk results. "However," to quote Berl and Peterson (*4*) "since the milks" (used by Dornbush et al.) "showed a variation of about 15 percent for the corresponding seasons in the 2 years, this difference is not significant."

Possibly a word should be said here regarding the relative potencies of the butters and milks of the two breeds of cows for which the results are given in table 30. On the average, 18 percent of the vitamin A potency of the Holstein milk and butter was due to carotene, whereas this figure for the Jersey milk and butter was 36 percent. Vitamin A potencies, as given in table 30 and generally throughout this report, are based on the relative effectiveness of carotene and vitamin A in promoting rat growth. The criteria on which human requirements have been based are quite different, and the results of Booher, Callison, and Hewston (*6*) indicate that 1 I. U. of carotene is only 50 or 60 percent as effective as is a unit of vitamin A in cod-liver oil in supporting normal dark adaptation—the criterion used by these workers in determining human requirements. This was under conditions where practically the total requirement was fed either as carotene or

[7] This distribution is used at the suggestion of Benjamin Bennett, Bureau of Agricultural Economics, United States Department of Agriculture.

vitamin A. There apparently are no results with the human that bear on the use of mixtures of vitamin A and carotene, like those that occur in butter and milk, as compared with diets supplying the requirement as either carotene or vitamin A.

It is, therefore, doubtful at present whether, in considering the human use of butter, one should or should not allow for the relative carotene and vitamin A contents in comparing the butters and milks from Jersey and Holstein cows. One might also question whether the groups of cows were large enough, whether adequate allowance was made for the increase in fat content with their advance in lactation, etc., to permit any general conclusion relative to the effect of breed on the vitamin A potency of the milk for human use.

DISCUSSION OF ANALYTICAL PROCEDURES USED IN THIS STUDY

Physiological Significance of Analytical Results

The antimony trichloride method was used for the determination of vitamin A, the procedure of Koehn and Sherman (*19*) being modified to use with butter. The carotene was separated from the other pigments in the butter by washing its solution in Skellysolve B with either 92-percent methyl alcohol or 94-percent diacetone alcohol.

From time to time referee samples of butter were analyzed by the laboratories participating in the work. The reports on these referee samples revealed very disturbing differences between the results of different laboratories analyzing the same samples of butter. This condition improved somewhat as the work progressed. The standard deviation of the individual results and the standard error of the mean of all the results on the last two referee samples are shown in table 31.

Table 31.—*Standard deviation of result from a single laboratory and standard error of the mean of all results on the last 2 referee samples of butter*

Sample No., and number of laboratories reporting	Mean of results reported	Standard deviation of single result	Standard error of the mean
	µg. per gram		
Vitamin A:			
No. 1 (15 laboratories)	4.22	0.38	0.10
No. 2 (15 laboratories)	6.49	.50	.13
Carotene:			
No. 1 (17 laboratories)	5.61	.65	.16
No. 2 (17 laboratories)	3.89	.58	.14

Although the random errors between the results reported by the various laboratories—which errors may, of course, have been systematic in the use of the method within the individual laboratory—were considerable, the standard error of the mean of all the results from these laboratories was certainly not disconcerting. There may, of course, have been some systematic error inherent in the method itself, which may have affected all of the results in common. To test this question and to throw light on the nutritional significance of the butter results, members of the Technical Committee, using the

methods recommended, determined the vitamin A and carotene in six butters. The vitamin A potencies of the same six butters were determined biologically at the Alabama Agricultural Experiment Station.

There is, of course, an error of considerable magnitude in such experiments; but when the carotene and vitamin A as chemically determined in these butters were expressed in I. U. as in this report, 1 I. U. in the butter had a biological potency well within the limit of error of 1 I. U. of beta-carotene—in terms of which the potency of vitamin A is defined at present.

Koehn of the Alabama Agricultural Experiment Station carried out the biological assays on these butters; Koehn, Palmer, Peterson, and Guilbert of the Alabama, Minnesota, Wisconsin, and California stations, respectively, determined the vitamin A in them by the chemical methods recommended; and results with the carotene method were reported for all samples by Koehn and Zscheile of the Alabama and Indiana stations, respectively. The results of this work are shown in tables 32 and 33.

The International Units shown for these butters in table 32 (columns 10, 12, and 14) and in expressing the potencies of the doses of butter fed in the bioassays (table 33, column 3) are calculated as indicated in table 32, footnote 5. In each assay the butter was fed at two levels and the carotene at one level, i. e., 3.33 I. U. for the carotene.

The results in table 32, columns 5 to 9, like those in table 31, show again the agreement that was obtained when the methods recommended by the Technical Committee were used by several laboratories to determine vitamin A in samples of the same butters.

TABLE 32.—*Chemical results with the 6 butters that were also assayed biologically for vitamin A* [1]

Sample No.	Carotene per gram in butterfat assayed by—			Vitamin A per gram in butters assayed by [3]—					Calculated potency per gram of butter [5]				
	Koehn (Ala.)	Zscheile (Ind.)[2]	Average	Palmer (Minn.)	Peterson (Wis.)	Koehn (Ala.)	Guilbert (Calif.)[4]	Average	Due to carotene		Due to vitamin A		Total
	µg.	*µg.*	*µg.*	*µg.*	*µg.*	*µg.*	*µg.*	*µg.*	*I. U.*	*Percent*	*I. U.*	*Percent*	*I. U.*
1	8.27	7.42	7.84	6.23	6.81	6.56	6.74	6.58	13.07	33.2	26.32	66.8	39.39
2	3.31	3.06	3.18	4.54	4.92	5.03	4.82	4.83	5.3	21.5	19.32	78.5	24.62
3	8.98	8.98	8.98	6.78	6.94	7.61	7.28	7.15	14.97	34.3	28.60	65.7	43.57
4	6.74	6.93	6.84	4.85	4.88	5.00	4.54	4.82	11.4	37.2	19.28	62.8	30.68
5	5.45	5.40	5.42	4.56	4.63	4.77	4.84	4.70	9.03	32.4	18.80	67.6	27.83
6	2.98	3.06	3.02	3.38	3.70	3.94	3.47	3.62	5.03	25.8	14.48	74.2	19.51
Average				5.06	5.31	5.48	5.28	5.29		30.7		69.3	
Standard error [6]								±.086					

[1] These same 6 butters were assayed biologically for vitamin A and the results are given in table 33.
[2] Supplementary report to Dr. Koehn, dated June 17, 1942.
[3] Not corrected for loss of about 7 percent in analysis.
[4] Supplementary report to Dr. Koehn from Dr. Guilbert.
[5] Used 0.6 microgram of the carotene and 0.25 microgram of the vitamin A as equal to 1 I. U.—i. e. 1 microgram of vitamin A, uncorrected for loss in analysis, is taken as equal to 4 I. U. This would be equivalent to taking 3.7 I. U. as equal to 1 microgram of vitamin A in the original butter, assuming that the same loss of vitamin A occurred in the analysis of the butter that was found to occur when vitamin A was added to butter.
[6] Standard error of the mean of the 4 means obtained for all samples in each of the 4 laboratories.

TABLE 33.—*Biological results with 6 butters that were also assayed chemically for vitamin A* [1]

Butter No.	Doses of butter fed daily	Vitamin A fed in butter or carotene [2]	Growth of rats: 3 weeks	4 weeks	5 weeks	6 weeks	7 weeks
	Gm.	*I. U.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Butter No. 1:							
Rats fed butter	0.0951	3.74	42	57	71	81	92
	.0475	1.87	21	33	45	52	57
Rats fed carotene [3]		3.33	32	43	51	54	62
Butter No. 2:							
Rats fed butter	.1632	4.01	45	64	80	94	105
	.0787	1.94	25	36	45	51	58
Rats fed carotene		3.33	52	67	85	96	102
Butter No. 3:							
Rats fed butter	.0810	3.53	33	48	64	77	91
	.0420	1.83	23	31	50	60	73
Rats fed carotene		3.33	25	37	59	70	89
Butter No. 4:							
Rats fed butter	.1224	3.76	44	62	78	90	97
	.0652	2.00	25	37	49	59	63
Rats fed carotene		3.33	42	60	70	82	90
Butter No. 5:							
Rats fed butter	.1224	3.40	38	54	68	82	93
	.1061	2.95	32	53	64	77	88
Rats fed carotene		3.33	35	52	67	79	90
Butter No. 6:							
Rats fed butter	.2060	4.02	40	53	62	69	76
	.1750	3.41	35	51	62	71	77
Rats fed carotene		3.33	32	46	56	67	75
Averages:							
All butter groups		3.04	33.6	44.2	61.5	71.9	80.8
8 butter groups, receiving a dosage of 2.95 to 4.02 I. U.		3.60	38.6	55.2	68.6	80.1	90.0
All carotene groups		3.33	36.3±3.8	50.8±4.6	64.7±5.0	74.0±5.9	84.7±5.7
5 carotene groups [4]		3.33	37.2±4.6	52.4±5.2	67.4±5.1	78.8±5.1	89.2±4.3
Average growth (gm. per I. U.):							
All butter groups			11.0	15.9	20.2	23.7	26.6
8 butter groups, receiving a dosage of 2.95 to 4.02 I. U.			10.7	15.3	19.0	22.2	25.0
All carotene groups			10.9	15.3	19.4	22.2	25.4
5 carotene groups [4]			11.2	15.7	20.2	23.7	26.8

[1] These same 6 butters were analyzed chemically for vitamin A and the results are given in table 32.
[2] Each carotene-fed group was fed 2 micrograms of carotene containing 3.33 I. U. of vitamin A.
[3] The purity and stability of the carotene fed to this group of rats was not so carefully controlled as that of the carotene fed to the other 5 groups, and the Alabama workers suggest that it be omitted in considering the results of these assays.
[4] The 5 groups in which the purity and stability of the carotene was carefully controlled are included in these averages (see footnote 3 above).

In considering the data in table 33 (on the average growth of the rats in grams per I. U. of vitamin A in the diet) one is certainly much more impressed by the extent of agreement between the results with the butter and the standard carotene than by the differences. This is true whether one uses, in this comparison, the average growth for all the butter-fed groups (average dose 3.04 I. U. of vitamin A) or the average for the eight butter-fed groups that received an average of 3.6 I. U. of vitamin A. The agreement is particularly impressive when one considers the magnitude of the standard error of the mean growths for the carotene-fed groups and the fact that an error of similar nature occurs in the mean growths of the butter-fed groups. It should, however, be pointed out that Dr. Salmon of the Alabama Agricultural Experiment Station, as a member of the Technical Committee, considers that the difference in the 7 weeks' recovery period (between the average growths in grams per International Unit of vitamin A

fed the five carotene groups and some of the butter-fed groups that received the larger doses of butter) may suggest that possibly the International Units in the doses of butter in table 33 have been calculated somewhat too high.

It should also be pointed out that these expressions of average growth and of growth per International Unit of vitamin A in the butter imply a straight line relation between the various levels of dose and growth—a relation which certainly did not exist in these assays. For instance, using the average growths for the 4- and 5-week recovery periods, doses of 2, 3, and 4 I. U. corresponded respectively to growths per International Unit of 21.4, 18.7, and 16.5 grams. The percentage increase in dose with the higher levels of dosage was about twice the corresponding percentage increase in growth.

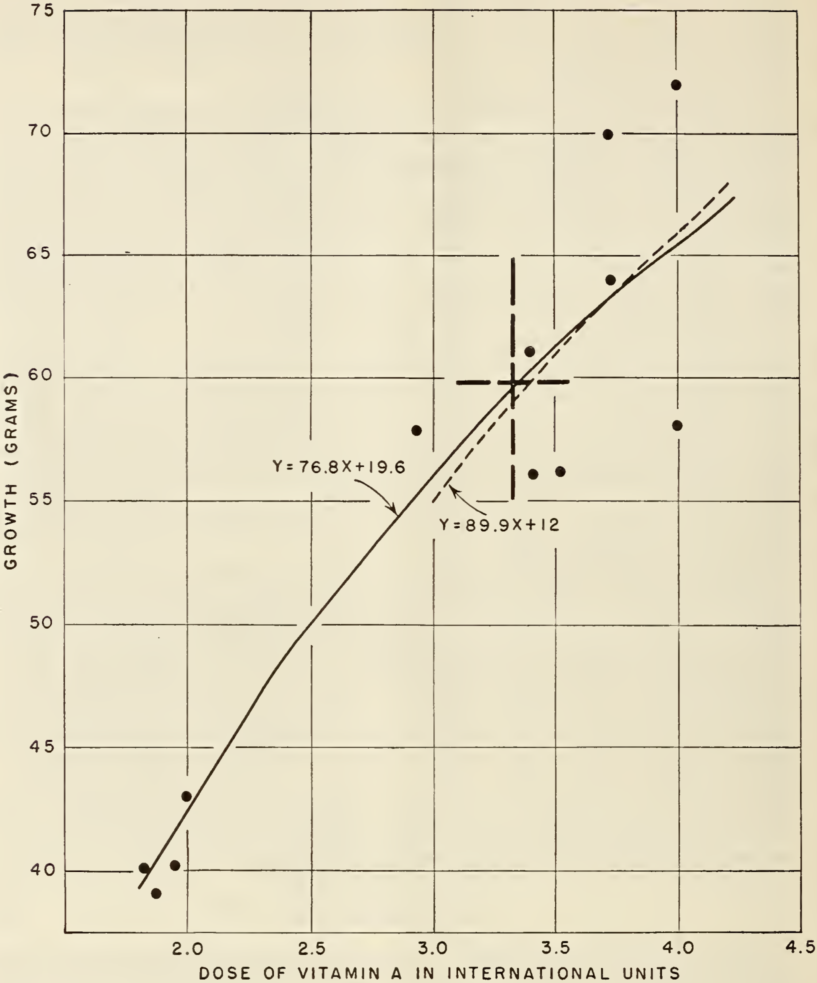
In such comparisons as the above, it is difficult to allow for the differences in dose-growth relationships. For instance, although the carotene dose is about midway between the average dose for all the butter-fed groups and the average for the eight groups fed 2.95 to 4.02 I. U. in butter, the growths of these two sets of butter-fed groups may be far from comparable with the growth of the groups on carotene. The difficulty of allowing for the differences is possibly lessened by using the eight butter groups that were fed the larger doses of vitamin A, but it is not eliminated.

On account of these difficulties it would seem that the average dose-growth response relation for these butters might better be expressed by an equation calculated as described by Coward (*9*, p. 21). As the average rate of growth in these assays was greatest during the fourth week, declined somewhat in the fifth week, and was materially lower in the sixth and seventh weeks, it might be best to calculate this equation for this relation by using the average for the growths given in table 33 for 4 and 5 weeks. These calculations give equation 1 when all groups on butter are used, and equation 2 when only the eight groups on butter are used in which the doses of vitamin A in the butter were 2.95 to 4.02 I. U., inclusive. In these equations Y is the growth and X is the log of the daily dose.

$$Y=76.8X+19.6 \tag{1}$$
$$Y=89.9X+12.0 \tag{2}$$

Figure 2 shows graphically the actual dose-growth response relation found with each of these groups of data for the vitamin A with each level of butter fed, the average dose-growth response relation for the vitamin A in these butters as expressed by these equations, and the average and standard error of the growth of the rats receiving 3.33 I. U. of beta-carotene.

According to equation 1, 3.33 I. U. in the butter gave an average growth of 59.4 grams; and according to equation 2, it gave an average growth of 59 grams; whereas the average growth of the rats fed 3.33 I. U. in carotene was 59.9 ± 5.0 grams using the five groups with which the purity and stability of the carotene were more carefully controlled (as in figure 2), or 57.8 ± 4.7 grams using the average of all six groups that were fed carotene. Similar calculations have been made using other recovery periods and other groupings of the rats (table 34). In no case is the mean growth on 3.33 I. U. in the butter significantly different from that actually found for 3.33 I. U. fed as beta-carotene.



Butter groups.

Intersection designates mean growth (average 4 and 5 weeks) of 5 groups fed 3.33 I. U. of carotene. Length of vertical indicates magnitude of standard error of this mean.

Average growth of all groups of butter-fed rats as expressed by equation $Y=76.8X+19.6$ for average growth of 4 and 5 weeks.

Average growth of 8 groups of butter-fed rats, receiving 2.95–4.02 I. U. of vitamin A daily, as expressed by equation $Y=89.9X+12$ for average growth of 4 and 5 weeks. (X=log of dose and Y=growth)

FIGURE 2.—Dose-growth response relation with six butters assayed chemically and biologically

In fact, the agreement is exceedingly good between the average growth per I. U. as thus arrived at in the butter and as fed as carotene; but, although the standard error of the mean growth of the groups on the carotene (an error which, as pointed out above, also occurs in the equations for the mean growth-dose relationship for the butters) is not unusually large for this type of experiment, these errors clearly disturb the significance of such an agreement. (See fig. 2.)

TABLE 34.—*Comparison of mean growth as calculated for 3.33 I. U. of vitamin A in butter and mean growth on 3.33 I. U. fed as carotene*

Item of comparison	Mean growth calculated for 3.33 I. U. in butter [1]	Mean growth found for 3.33 I. U. fed as carotene	
		Using results of 5 assays [2]	Using results of 6 assays
	Gm.	*Gm.*	*Gm.*
Growth, 4 weeks; using all groups fed butter	52.0	52.4±5.24	50.8
Growth, 4 weeks; using only the lower dose on each butter	53.4	52.4±5.24	50.8
Growth, average of 4 and 5 weeks; using all groups fed butter	59.4	59.9±5.00	57.8
Growth, average of 4 and 5 weeks; using 8 groups fed 2.95 to 4.02 I. U. in butter	59.0	59.9±5.0	57.8
Growth, average of 5, 6, and 7 weeks; using all groups on butter	76.4	78.5±4.7	74.5
Growth, 7 weeks; using all groups on butter	88.6	89.2±4.3	84.7
Growth, 7 weeks; using 8 groups fed 2.95 to 4.02 I. U. in butter	88.8	89.2±4.3	84.7

[1] For weeks 5 to 7 with butter No. 6 (table 33) the growth on the larger dosage of butter was not higher than on the lower dose.
[2] In 5 of the assays the purity and stability of the carotene was more carefully controlled. Only these 5 are included in the averages in this column.

Results in accord with those for butter were also obtained when samples of the 1,700 and 3,000 U. S. P. unit oils were similarly analyzed by the chemical method recommended for use with the butter. In early work, some members of the Technical Committee calibrated their spectrophotometers or photoelectric colorimeters against samples of one of these oils. The results from these various laboratories did not agree well. The Bureau of Dairy Industry laboratory at Beltsville found that the $E^{1\text{ USP unit/ml.}}_{1\text{ cm.}}$ 620 millimicrons for various samples of the 1,700 U. S. P. oil varied. The maximum values for the two standard oils were about 0.124 and 0.120, respectively, as calculated on the basis of their rated potencies; whereas the $E^{1\text{ mcg./ml.}}_{1\text{ cm.}}$ 620 millimicrons value for samples of vitamin A alcohol, determined directly or calculated from its acetate, was about 0.51. A microgram of vitamin A as determined in the oil, therefore, would be equivalent to 4.1 or 4.25 U. S. P. units, respectively. This is without correcting for any losses of vitamin A that may have occurred in the analyses of these oils. These results were obtained with samples of these oils which, when freshly opened, had $E^{1\text{ percent}}_{1\text{ cm.}}$ 325 millimicrons values of 0.87 and 1.50 (or 1.54 when read on another spectrophotometer). These values are not greatly different from the maximum values of 0.90 and 1.58 which Nelson [8] and Morton (*22*, p. 367) give

[8] According to communication from Dr. E. M. Nelson, Food and Drug Administration, Federal Security Agency.

for them, respectively. Morton's value would seem to have represented the $E_{1\,\text{cm.}}^{1\,\text{percent}}$ 325 millimicrons value of the 3,000 U. S. P. unit oil when it had a potency equivalent to 3,000 International Units (*16*).

A relation similar to that above with the antimony trichloride reagent could not be expected as a result of a similar comparison of the $E_{1\,\text{cm.}}^{1\,\text{mcg./ml.}}$ 325 millimicrons for crystalline vitamin A with the $E_{1\,\text{cm.}}^{1\,\text{USP unit/ml.}}$ 325 millimicrons values for these U. S. P. standard oils, as materials other than vitamin A affect this absorption of these oils even when they are previously saponified. This was shown with the 1,700 U. S. P. unit oil in work done in the Bureau of Dairy Industry laboratory at Beltsville.

On the other hand, Dr. Salmon of the Alabama Experiment Station found, in calibrating an Evelyn machine, that he obtained the same L 620 millimicrons value for 0.3 microgram of vitamin A alcohol and 1 U. S. P. unit in the 3,000 U. S. P. unit oil. The sample of vitamin A alcohol that he used apparently had an $L_{1\,\text{cm.}}^{1\,\text{percent}}$ 620 millimicrons value of 3,900; and it may be pointed out that according to Baxter and Robeson (*3*) and Dann and Evelyn (*10*) this would correspond to an $E_{1\,\text{cm.}}^{1\,\text{percent}}$ 328 millimicrons value of about 1,600 for this alcohol, whereas values close to 1,800 are reported for preparations of this alcohol that are now readily obtainable.

Possibly not all the factors that affect the relative physiological utilization of carotene and vitamin A are known; additional factors and conditions are being recognized from time to time which affect this relation. All that can be said now, from the data in tables 32, 33, and 34 and figure 1, is that these data accord with and, insofar as such data can, they do tend to support the use of the chemical methods and methods of calculation used in arriving at the potency of these butters; and the results with the U. S. P. reference oils tend further to confirm this conclusion. It is also certainly obvious that these methods are far more precise and possibly far more accurate than our knowledge of the vitamin A requirements of the human or of any domestic animal.

By the time the work on the present survey was started with the methods recommended by the Technical Committee, Kemmerer and Fraps had started their work on the vitamin A potency of butters sold on the markets in Texas (see p. 25); and it may be well to compare results by their methods (*13*) with results obtained by the methods recommended by the Technical Committee. The analytical methods used by the Texas workers were quite different from those recommended by the Technical Committee—particularly in the case of vitamin A itself. The Texas workers determined the vitamin A by means of the absorption of light at wave length 328 millimicrons by a solution of the nonsaponifiable fraction of the butter. This procedure had previously been used in several investigations at the University of Wisconsin and in England. Both sets of workers had presented evidence that the curve showing the relative absorption of light at different wave lengths by this nonsaponifiable fraction did not correspond to that of vitamin A; and at least one unsuccessful attempt

had been made to separate the vitamin A from the other materials in this fraction that distorted this curve, and to determine what proportion of the light absorbed at 328 millimicrons was actually due to vitamin A. As a result of the study of a large series of butters (28 butters) in which they determined the vitamin A and carotene by their methods and biologically, Fraps, Kemmerer, and Meinke (*13*) arrived at the following equation for calculating the potency of butter:

$$\text{I. U.}=(S-0.5)4+1.7C$$

in which S and C are respectively the vitamin A and carotene in micrograms as they determined them in the butter by their physicochemical methods.

When the present survey was started the Texas workers analyzed two of the referee samples sent to the various State stations by the Technical Committee. At that time certain stations were not yet familiar—and were obviously having difficulty—with the recommended method. The results reported by such stations will be omitted in comparing the method used by the Texas workers with that recommended by the Technical Committee. Also, it is obvious that the results in micrograms obtained by the two methods, especially in the case of vitamin A itself, would not be strictly comparable. Since, however, the Texas workers derived their method of calculating their results in International Units from a very extensive comparison of biological assays with their chemical results, it may be of interest to compare the potencies of these two referee samples of butter as thus calculated from the chemical results reported by the Texas group with the potencies calculated as in tables 32 and 33 from the results obtained by the methods recommended by the Technical Committee, or with potencies similarly calculated from these results except that the results for vitamin A itself are corrected for the loss of it that occurs in analysis. These comparisons are shown in table 35.

TABLE 35.—*Comparison of method for the determination of the vitamin A potency of butter used in Texas with that recommended by the Technical Committee*

Referee sample No.	Technical Committee method				Results by Texas workers with their own method		
	Carotene per gram of butter	Vitamin A per gram of butter	Potency per gram: Not corrected for loss in analysis [1]	Potency per gram: Corrected for loss of vitamin A in analysis [2]	Carotene per gram of butter [3]	Vitamin A per gram of butter [3]	Potency per gram of butter [3]
	μg.	*μg.*	*I. U.*	*I. U.*	*μg.*	*μg.*	*I. U.*
1	[4] 4.44	[4] 5.15	28.0	29.6	4.8	6.0	30.2
2	[5] 8.94	[6] 6.33	40.2	42.1	10.2	7.4	44.9

[1] 0.6 microgram of carotene=0.25 microgram of vitamin A=1 I. U.
[2] 0.6 microgram of carotene=0.25 microgram of vitamin A=1 I. U., the vitamin A itself being corrected on the basis of the recovery of 93 percent of it in analysis.
[3] The results in micrograms are as reported. The potency was calculated from these reported figures, using I. U.=(S−0.5)4+1.7C (see text).
[4] Average of results from 8 laboratories.
[5] Average of results from 11 laboratories.
[6] Average of results from 9 laboratories.

Evidently the methods used by the Texas workers gave potencies somewhat higher than those obtained by either of the methods used in calculating the potencies of these two butters from the results obtained by the chemical methods recommended by the Technical Committee. The differences were not significant, however, when the results with the Technical Committee method were corrected for the vitamin A lost in analysis. These results in table 35 indicate that the potencies obtained for butters by Kemmerer and Fraps in Texas may be comparable with those in this report expressed in International Units "corrected for loss of vitamin A in analysis." However, only two referee samples were compared, and these contained no artificial coloring matter.

Zscheile, as a member of the Technical Committee, also published a method for the determination of vitamin A and carotenoids in butterfat (*27*) in which the vitamin A was likewise determined in micrograms from the spectrophotometric reading of a solution of the nonsaponifiable fraction at the wave length of 326 millimicrons. With the six butters for which the results are given in tables 32 and 33, this method gave results based on the total readings at 326 millimicrons that were on an average about 1.28 times those obtained by the method recommended by the Technical Committee; and this relation appeared to be close to constant with these six butters. The method was not used in this survey, as the Purdue workers encountered difficulty in correcting for artificial coloring when it was added to butter. They say: "Since the presence of azo dyes does not interfere seriously, the antimony trichloride reaction is the preferred physicochemical method available for butters containing such dyes."

Caldwell and Parrish (*7*) also studied the effect of light on the stability of the Carr-Price color in the antimony trichloride method of determining the vitamin A in butter.

Converting the Micrograms of Carotene and Vitamin A into International Units

Despite the results reported in the preceding section one may well question the use of 4 I. U. (equivalent to 3.7 I. U. when the micrograms of vitamin A are first corrected for loss in analysis) as equal to 1 microgram of vitamin A. Some workers might be inclined to use 3.3 I. U., the average of the results of Mead, Underhill, and Coward (*21*) and more recently quite specifically endorsed by Morton (*22*) as probably expressing the potency of vitamin A with the rat; or 4.3 ± 0.39 U. S. P. units, the average of the results reported by Baxter and Robeson (*2*). The problems involved in such a choice have been reviewed in recent years (*11, 15, 22*).

Also with the carotene as here determined in butter there may be some error—possibly small—in the assumption that "all" of the pigment in this fraction has the vitamin A potency of beta-carotene. The proportion of alpha-carotene in butterfat is undoubtedly negligible unless alpha-carotene occurs in unusual amounts in the feed of the cow. Pigments other than beta-carotene do occur to some extent in this carotene fraction and it is an open question as to what extent these other pigments may be artifacts, and as to what biological activity if any they represent (see Zscheile, et al. (*28*)), but error from this source is probably relatively small and may be disregarded.

It may be well, however, to consider the results of Mead, Underhill, and Coward (*21*) and of Baxter and Robeson (*2*) insofar as they may bear on the calculation of the potency of our butters from the analytical results. The former workers used beta-carotene as a standard; the latter, the 1,700 U. S. P. unit oil. Mead, Underhill, and Coward assayed the vitamin A potency of two esters, the vitamin A anthraquinone-2-carboxylate and the vitamin A-2-naphthoate. No evidence is given that the vitamin A in these esters would have the same biological potency as equivalent amounts of vitamin A as it occurs in nature as the alcohol or as an ester of an aliphatic acid; this was assumed.

These workers made three assays with each ester. The anthraquinone-2-carboxylate had a purity corresponding to an A alcohol with an $E^{1\text{ percent}}_{1\text{ cm.}}$ 328 millicrons value of 1,510. The results of the individual assays correspond in round numbers to potencies of 1.9, 2.8, and 5.1 International Units per microgram of the vitamin A in the ester. With the other ester, which was somewhat purer and with which they obtained results in better agreement, the potencies found correspond to 2.9, 3.8, and 3.8 I. U. per microgram of the vitamin A alcohol in it; but with another preparation of this same ester, which Baxter and Robeson report to have been similar in purity, Baxter and Robeson obtained, as an average of four assays, results that correspond to 5.29 ± 0.24 U. S. P. units per microgram of the vitamin A alcohol in the ester. Baxter and Robeson report that their figures "may be slightly altered in a forthcoming paper." But they also say that "The crystalline aliphatic esters of vitamin A had substantially the same equivalent biological potency as crystalline vitamin A." Regarding the factor for converting weights to potency units they say: "The factor for vitamin A-naphthoate was significantly higher than that of the aliphatic esters." As a result of nine bioassays of the acetate, eight of the palmitate, and eight of the vitamin A alcohol itself, Baxter and Robeson report results corresponding to 4.04 ± 0.23, 4.23 ± 0.31, and 4.30 ± 0.39 U. S. P. units respectively per microgram of vitamin A.

There is evidently considerable area for speculation regarding the significance of the results of Mead, Underhill, and Coward; but one might also feel uncertainty regarding the significance of the results reported by Baxter and Robeson. At one time the unit in the 3,000 U. S. P. unit standard oil was on an average equivalent to 1 I. U. (*16*). The question is: Was this true with the samples of the 1,700 U. S. P. unit oil used by Baxter and Robeson? Harris,[9] who conducted these assays, of which the results were reported by Baxter and Robeson, has been kind enough to inform us regarding the samples of this oil that he used. He says: "Our values for $E^{1\text{ percent}}_{1\text{ cm.}}$ 328 millimicrons have varied all the way from 0.79 to 0.89 (average 0.85) when received. Those samples which we use for bioassay standards are opened immediately upon receipt, carefully divided into six or seven portions, and each of these sealed under nitrogen in separate brown-

[9] Communication from Philip L. Harris of Distillation Products, Inc., to C. A. Cary, April 14, 1943.

glass containers. One of these is opened at weekly intervals . . ."

Similar variations in the $E^{1\text{ percent}}_{1\text{ cm.}}$ 325 millimicrons values of samples of the 1,700 U. S. P. unit oil, and corresponding variations in the $E^{1\text{ percent}}_{1\text{ cm.}}$ 620 millimicrons values obtained with them with the antimony trichloride method as here recommended for butter, have been noted in this survey by the Bureau of Dairy Industry laboratory with samples of the 1,700 U. S. P. unit oil shipped packed in dry ice from storage in Philadelphia and analyzed immediately upon receipt at Beltsville; and the Bureau of Dairy Industry laboratory obtained from other laboratories samples of this oil that were considered usable and one of which was in use that varied from 0.70 to 0.87 in their $E^{1\text{ percent}}_{1\text{ cm.}}$ 325 millimicrons value, and from 0.106 to 0.125 in their $E^{1\text{ percent}}_{1\text{ cm.}}$ 620 millimicrons value when analyzed with the antimony trichloride reagent. Although the 3,000 U. S. P. unit oil when first assayed by a number of laboratories in this country was found to have a potency of 3,000 I. U. per gram, the average of later assays in England against the beta-carotene standard gave on an average only about 2,600 I. U. per gram. This difference has been attributed to the decomposition of the vitamin A in this oil, and Morton (*22*) suggests that this decrease in potency is correlated quantitatively with a drop in the $E^{1\text{ percent}}_{1\text{ cm.}}$ 325 millimicrons value of this oil.

Recently Callison and Orent-Keiles (*8*) reported assays in which the same material in the same assay was compared with both beta-carotene and the 1,700 U. S. P. unit oil fed to comparable groups of rats. These workers obtained differences which they attribute to partial decomposition of the vitamin A in the samples of the 1,700 U. S. P. unit oil that they used; but the samples of the oils when first tested had $E^{1\text{ percent}}_{1\text{ cm.}}$ 325 millimicrons values of only about 0.70 and 0.77 and $E^{1\text{ USP unit/ml.}}_{1\text{ cm.}}$ 620 millimicrons values of only about 0.107 and 0.108, and showed further evidence of deterioration with subsequent tests. Although it is obvious that these standard oils are unstable and that tests should be made from time to time to determine whether or not the vitamin A in them has undergone substantial destruction, it is a question as to what extent this may be the explanation of the differences between the average of the results of Mead, Underhill, and Coward and those reported by Baxter and Robeson.

If the average $E^{1\text{ percent}}_{1\text{ cm.}}$ 328 millimicrons value of 0.85, reported by Harris,[10] represents the actual average of the oils fed, it would correspond to a decrease in the potency of the 1,700 U. S. P. unit standard of possibly not more than about 6 percent—if the decomposition is taken as proportional to this value. If we allow for this loss and assume, further, that the 1,700 U. S. P. unit oil originally had a potency equal to 1,700 I. U., the 4.3 U. S. P. unit reported by Baxter and Robeson as equivalent to 1 microgram of vitamin A might be

[10] See footnote 7, p. 33.

taken as equivalent to about 4.04 I. U. This is under circumstances where no analytical losses would be involved; this would be equivalent to using 4.3 International Units per microgram of vitamin A in butter as here reported in micrograms uncorrected for the loss in analysis, instead of the 4 I. U. as used under these circumstances in this report. But some uncertainty regarding the expression of these results in International Units will exist until further work is done in which these various crystalline vitamin A products are assayed biologically directly against beta-carotene of known purity using a basal vitamin A-free diet that is known to be "complete" in all respects, except vitamin A, and is known to promote the optimum use of both carotene and vitamin A.

The biological results with the butter (previous section of this report, and tables 32 and 33), the relation between the $E^{1\ \mathrm{mcg./ml.}}_{1\ \mathrm{cm.}}$ 620 millimicrons value for the more potent preparations of vitamin A alcohol and the maximum $E^{1\ \mathrm{USP\ unit/ml.}}_{1\ \mathrm{cm.}}$ 620 millimicrons values for the U. S. P. standard oils, and the results of Baxter and Robeson would favor the use of approximately 4 International Units as equivalent to 1 microgram of vitamin A. However, the use of this factor is quite arbitrary; and, as our knowledge develops, it may be desirable to recalculate—or to calculate in various ways for various purposes—the potencies given in this report. The data are included that will permit this recalculation.

LITERATURE CITED

(1) Ashworth, U. S., McGregor, M., and Bendixen, H. A.
1945. the carotene and vitamin a content of creamery butter produced in washington. Wash. Sta. Bul. 466, 8 p., illus.

(2) Baxter, J. G., and Robeson, C. D.
1942. crystalline aliphatic esters of vitamin a. Jour. Amer. Chem. Soc. 64: 2407–2410.

(3) ——— and Robeson, C. D.
1942. crystalline vitamin a. Jour. Amer. Chem. Soc. 64: 2411–2416.

(4) Berl, S., and Peterson, W. H.
1943. determination and content of carotene and vitamin a in wisconsin butter. Jour. Nutr. 26: 527–538.

(5) ——— and Peterson, W. H.
1945. distribution of carotene and vitamin a in butter-making. Jour. Dairy Sci. 28: 103–107.

(6) Booher, L. E., Callison, E. C., and Hewston, E. M.
1939. an experimental determination of the minimum vitamin a requirements of normal adults. Jour. Nutr. 17: 317–331.

(7) Caldwell, M. J., and Parrish, D. B.
1945. effect of light on the stability of the carr-price color in the determination of the vitamin a in butter. Jour. Biol. Chem. 158: 181–186.

(8) Callison, E. C., and Orent-Keiles, E.
1945. standards in vitamin a assays. Indus. and Engin. Chem., Analyt. Ed., 17: 378.

(9) Coward, K. H.
1938. the biological standardization of the vitamins. 227 pp. London.

(10) Dann, W. J., and Evelyn, K. A.
1938. the determination of vitamin a with the photoelectric colorimeter. Biochem. Jour. 32: 1008–1017.

(11) Dornbush, A. C., Peterson, W. H., and Olson, F. R.
1940. the carotene and vitamin a content of market milks. Jour. Amer. Med. Assoc. 114: 1748–1751.

(12) Fieger, E. A., and Lewis, Harvye.
1944. the vitamin a content of milk and butter as related to the nature of pasture. La. Agr. Expt. Sta. Bul. 388, 22 pp., illus.

(13) Fraps, G. S., Kemmerer, A. R., and Meinke, W. W.
1941. relation of chemical analyses of butter to its vitamin a potency. Jour. Assoc. Off. Agr. Chem. 24: 731–735.

(14) Herrington, B. L.
1945. butter tested for vitamin a. Farm Res. N. Y. State Sta. 11 (4): 1, 6.

(15) Hickman, K.
1943. fat-soluble vitamins. Ann. Rev. Biochem. 12: 353–396.

(16) Hume, E. M. and Chick, H.
1935. the standardization and estimation of vitamin a. Med. Res. Council, Spec. Report Ser. No. 202, 61 pp. London.

(17) Jenness, R., and Palmer, L. S.
1945. vitamin a potency of creamery butter produced in minnesota. Jour. Dairy Sci. 28: 473–494.

(18) Kemmerer, A. R., and Fraps, G. S.
1943. the vitamin a potency of commercial butters sold in texas. Tex. Agr. Expt. Sta. Bul. 629, 12 pp.

(19) Koehn, C. J., and Sherman, W. C.
1940. THE DETERMINATION OF VITAMIN A AND CAROTENE WITH A PHOTOELECTRIC COLORIMETER. Jour. Biol. Chem. 132: 527–538.

(20) Krauss, W. E., Skinner, L., Hibbs, J. W., Armstrong, T. V., and Slatter, W. L.
1945. VITAMIN A POTENCY OF OHIO BUTTER. Ohio Agr. Expt. Sta. Bimo. Bul. 236, 157–163, illus.

(21) Mead, T. H., Underhill, S. W. F., and Coward, K. H.
1939. CRYSTALLINE ESTERS OF VITAMIN A. Biochem. Jour. 33: 589–600.

(22) Morton, R. A.
1942. FAT-SOLUBLE VITAMINS. Ann. Rev. Biochem. 11: 365–390.

(23) Parrish, D. B., Martin, W. H., Atkeson, F. W., and Hughes, J. S.
1946. THE VITAMIN A AND CAROTENE CONTENT OF MARKET BUTTER PRODUCED IN KANSAS. Jour. Dairy Sci. 29: 91–99.

(24) Richardson, G. A., and Long, M. L.
1945. VITAMIN A CONTENT OF CALIFORNIA-PRODUCED BUTTER. Pacific Dairy Rev. 49: 12, 32.

(25) Theophilus, D. R., Stamberg, O. E., Bolin, D. W., and Hanson, H. C.
1945. VITAMIN A POTENCY OF IDAHO BUTTER. Idaho Agr. Expt. Sta. Cir. 102, 4 pp., illus.

(26) United States Department of Agriculture.
1942. AGRICULTURAL STATISTICS. 840 pp.

(27) Zscheile, F. P., Nash, H. A., Henry, R. L., and Green, L. F.
1944. DETERMINATION OF VITAMIN A AND CAROTENOIDS IN BUTTERFAT: COMPARISON OF DIRECT SPECTROPHOTOMETRY WITH FILTER PHOTOMETRY AND USE OF THE ANTIMONY TRICHLORIDE REACTION. Indus. and Engin. Chem., Analyt. Ed., 16: 83–85.

(28) ———, Henry, R. L., White, J. W., Jr., Nash, H. A., Shrewsbury, C. L., and Hauge, S. M.
1944. DETERMINATION OF VITAMIN A AND CAROTENOIDS IN BUTTERFAT: SPECTROSCOPIC CHARACTERISTICS OF BUTTERFAT FRACTIONS AND PROBLEMS INVOLVED IN BIOLOGICAL INTERPRETATIONS. Indus. and Engin. Chem., Analyt. Ed., 16: 190–193.